Research Paper

Wild ginseng cambial meristematic cells ameliorate hepatic steatosis and mitochondrial dysfunction in high-fat diet-fed mice

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Abstract

Objectives The aim of this study was to determine the protective mechanisms of wild ginseng cambial meristematic cells (CMCs) on non-alcoholic fatty liver disease in high-fat diet (HFD)-fed mice.

Methods Male C57BL/6 mice received either normal-fat diet or HFD for 10 weeks along with wild ginseng CMCs (75, 150 and 300 mg/kg) or vehicle (0.5% carboxyl methyl cellulose) by oral administration once a day. Triglyceride and total cholesterol contents were measured in liver and serum samples. Parameters for hepatic lipid metabolism and mitochondria biogenesis were assessed.

Key findings Treatment with wild ginseng CMCs markedly attenuated body weight, serum and hepatic lipid contents, and serum aminotransferase activity. While wild ginseng CMCs attenuated the increases in sterol regulatory element-binding transcription factor 1 (SREBP-1) and carbohydrate-responsive element-binding protein (ChREBP) expression, it enhanced the increases in carnitine palmitoyltransferase 1A (CPT1A) and peroxisome proliferator-activated receptor alpha (PPAR-α) expression. HFD decreased glutamate dehydrogenase activity and glutathione content, and increased lipid peroxidation, which were all attenuated by wild ginseng CMCs. Furthermore, wild ginseng CMCs enhanced mitochondrial biogenesis-related factors, including peroxisome proliferator-activated receptor-γ co activator 1α (PGC1α), nuclear respiratory factor 1 (NRF1) and mitochondrial transcription factor A (TFAM).

Conclusions Wild ginseng CMCs protect against HFD-induced liver injury, which prevents lipid accumulation and mitochondrial oxidative stress, and enhances mitochondrial biogenesis.

Introduction

Fatty liver disease is the build-up of excessive fat in the liver cells. Alcoholic fatty liver disease (ALD) and non-alcoholic fatty liver disease (NAFLD) are two main types of fatty liver disease. Although ALD is caused by chronic alcohol abuse, NAFLD could occur by many risk factors such as central adiposity, dyslipidemia, insulin resistance, type 2 diabetes mellitus, metabolic syndrome and genetic factors. NAFLD encompasses a wide spectrum of liver damage, ranging from steatosis alone to steatohepatitis, advanced fibrosis and cirrhosis.[1] Although the pathogenesis of NAFLD is not yet fully understood, the 'multiple hit process' is widely accepted, which provides a model that summarizes the complex factors and their interactions leading from free fatty acid metabolism to NAFLD. The initial hit involves excess hepatic lipid accumulation, which can progress to non-alcoholic steatohepatitis to a following hit, such as oxidative stress, lipid peroxidation and release of inflammatory mediators.[2]

Accumulating evidence indicates that hepatic mitochondria play a critical role in the development and pathogenesis of NAFLD.[3] Mitochondrial dysfunction in NAFLD affects hepatic lipid homeostasis and promotes reactive oxygen species (ROS) production, lipid peroxidation, cytokine release and cell death.[4,5] Mitochondrial biogenesis, the complex process promoting mitochondrial capacity through the growth and division of pre-existing

mitochondria, plays an essential role in maintaining mitochondrial homeostasis, and induction of mitochondrial biogenesis alleviates mitochondrial dysfunction.[6] In recent years, researchers have tried to determine the effects of drugs or active compounds in alleviating mitochondrial dysfunction via mitochondrial biogenesis in pathophysiological conditions. Dexamethasone decreased cholestatic liver injury by enhancing mitochondrial biogenesis, and curcumin prevented cerebral ischaemia reperfusion injury through increases in mitochondrial biogenesis in rats.[7,8]

Ginseng has been used in traditional herbal medicine for over 2000 years in Asian countries and has been mainly used as a tonic to invigorate weak bodies. Several studies have reported that ginseng possesses various pharmacological effects in experimental models of liver diseases, such as fatty liver disease, liver fibrosis, hepatic carcinoma and chemical-induced liver injury.[9] Furthermore, ginseng consumption led to a reduction in the expression levels of genes associated with lipid metabolism, as well as in the levels of leptin, insulin and adiponectin, all of which perform critical functions in the control of obesity.[10] Recently, wild ginseng was shown to restore mitochondrial dysfunction against oxidative stress and neurodegenerative diseases in in-vivo and in-vitro studies.[11,12] Ginseng, however, is rare and its rarity makes it very expensive. Furthermore, the yield of active components from ginseng can be highly variable depending on the field cultivation. Undifferentiated cambial meristematic cells (CMCs) are plant stem cells that function as vascular stem cells. These cells can be considered immortal due to their ability to theoretically divide an unlimited number of times, and this technique overcomes the several problems. We established a technique for the isolation and culture of innately CMCs. Isolation of CMCs from selected *P. ginseng* species provides a higher content of ginsenosides, such as ginsenosides Rb1, Rb2, Rc and Rd, than can be obtained from natural harvest.[13,14]

Therefore, this study investigated the protective mechanisms of wild ginseng CMCs against high-fat diet (HFD)-induced hepatic injury, with a particular focus on mitochondrial dysfunction and biogenesis. Our findings suggest the potential therapeutic strategy of wild ginseng CMCs to counteract to NAFLD through promoting mitochondrial function and eliminating oxidative stress.

Materials and Methods

Plant cell culture and HPLC analysis

Wild ginseng CMCs were obtained from the cambium of *P. ginseng* Meyer (wild ginseng; native to Kangwon Province, Korea, for >50 years) and authenticated by the Korea Association of Wild Ginseng Appraiser (Sungnam, Korea). Wild ginseng CMCs were cultured in two stages: (1) a

proliferation stage to obtain biomass in the 250-l bioreactor (Fermentec Co. Ltd, Cheongwon, Korea), and (2) a production stage to obtain secondary metabolites, such as ginsenosides. Wild ginseng CMCs underwent a cell habituation process for 1 year without growth regulators.[15] Wild ginseng CMCs obtained through this process were maintained for 40 months. After proliferation and production cultures, wild ginseng CMCs were heat-treated in a heater at 95°C for 48 h. After the heat treatment, wild ginseng CMCs were separated from the medium through vacuum filtration, and then the CMCs were freeze-dried. Freeze-dried wild ginseng CMCs were ground to a fine powder. A voucher specimen (U2(M9)PS7DT17) was deposited at the Unhwa Corp (Jeonju, Korea).

For HPLC analysis, heat-treated wild ginseng CMCs were ground to a fine powder, and an 80% methanol solution was added to 0.5 g of ground, heat-treated wild ginseng CMCs to achieve a volume of 10 ml. This solution was extracted for 2 h under ultrasonic waves, centrifuged and filtered through a 0.2-μm syringe filter for HPLC analysis. An Agilent HPLC 1260 DAD system and Agilent Zorbax Eclipse plus C18 column (4.6 × 100 mm, 3.5 μm) were used for the analysis of rare ginsenosides. The detection wavelength was 203 nm, the temperature of the column was 30°C, and the mobile phase was 0.05% trifluoroacetic acid (Daejung Chemicals & Metals, Siheung, Korea) in water and 0.05% trifluoroacetic acid in acetonitrile with a flow rate of 1 ml/min. The analysed chromatogram is shown in Figure 1. During analysis, the compounds were identified as ginsenosides Rg3, Rk1, Rg5, Rh2, Rk2, Rh3, PPD and unsaturated fatty acid through electrospray ionization - mass spectrometry (ESI-MS) and nuclear magnetic resonance (NMR). The contents of rare ginsenosides including Rg3, Rh2 and Rk1 + Rg5 in the dry cell of heat-treated wild ginseng CMCs were 25.5 mg/g, 13.0 mg/g and 29.0 mg/g, respectively.

Experimental groups

Male C57BL/6 mice weighing 22–24 g were obtained from Orient Bio Inc. (Seongnam, Korea). All experiments were approved by the Animal Care Committee of Sungkyunkwan University School of Pharmacy (SKKUIACUC-20140008), and the animals received care in compliance with the Principles of Laboratory Animal Care formulated by the National Institutes of Health (NIH Publication No.86-23, revised 1985). During 10 weeks, wild ginseng CMCs were dissolved in 0.5% carboxyl methyl cellulose (vehicle) and administered orally once a day. Silymarin (100 mg/kg) was also given as a positive control. The animals were fed with normal-fat diet (NFD, D12450B; Research Diets, Inc., New Brunswick, NJ, USA) or HFD (D12492; Research Diets, Inc.) for 10 weeks. The contents of NFD and HFD are provided in Table 1. Animals were
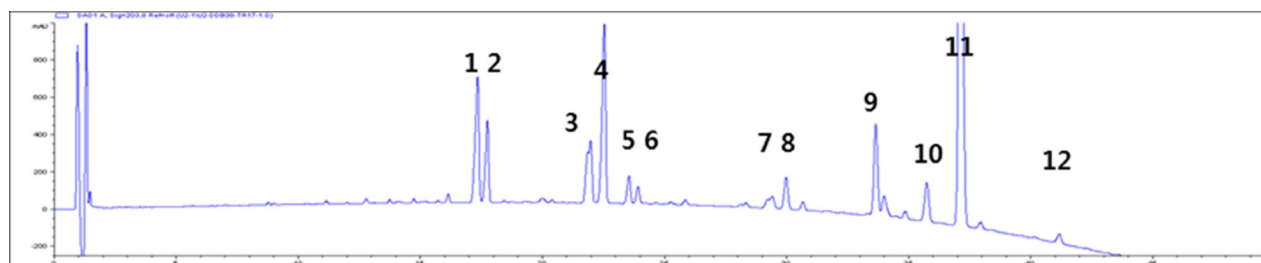
Figure 1 HPLC chromatogram of ginsenosides detected in heat-treated wild ginseng cambial meristematic cells. Numbers on the HPLC chromatogram show (1) ginsenoside 20(S)-Rg3, (2) ginsenoside 20(R)-Rg3, (3) ginsenoside Rk1, (4) ginsenoside Rg5, (5) ginsenoside 20(S)-Rh2, (6) ginsenoside 20(R)-Rh2, (7) ginsenoside Rk2, (8) ginsenoside Rh3, (9) linolenic acid, (10) ginsenoside 20(S)-PPD, (11) linoleic acid, and (12) oleic acid.

Table 1 Content of NFD and HFD

Ingredient	NFD		HFD	
	g	kcal	g	kcal
Casein, 80 mesh	200	800	200	800
L-cystine	3	12	3	12
Corn starch	315	1260	0	0
Maltodextrin 10	35	140	125	500
Sucrose	350	1400	68.8	275.2
Cellulose, BW200	50	0	50	0
Soybean oil	25	225	25	225
Lard	20	180	245	2205
Mineral mix, S10026	10	0	10	0
Dicalcium phosphate	13	0	13	0
Calcium carbonate	5.5	0	5.5	0
Potassium citrate, 1 H_2O	16.5	0	16.5	0
Vitamin mix, V10001	10	40	10	40
Choline bitartrate	2	0	2	0
FD&C yellow dye #5	0.05	0	0	0
FD&C blue dye #1	0	0	0.05	0
Total	1055.05	4057	773.85	4057

HFD, high-fat diet; NFD, normal-fat diet.

randomly separated into six groups (each group, *n* = 8–10): (a) NFD, (b) HFD, (c) HFD + wild ginseng CMCs 75 mg/kg (HFD 75), (d) HFD + wild ginseng CMCs 150 mg/kg (HFD 150), (e) HFD + wild ginseng CMCs 300 mg/kg (HFD 300), and (f) HFD + silymarin 100 mg/kg (HFD sily). Because there were no differences in any of the parameters between the vehicle-treated NFD and wild ginseng CMCs-treated NFD group, these groups were pooled and referred to as the NFD group. After 10 weeks of NFD or HFD feeding, the animals were sacrificed for liver and blood collection.

Lipid parameters

Serum triglyceride (TG) and total cholesterol (TC) concentrations were measured using an automatic hematology analyser (Korea Animal Medical Science Institute Co., Ltd, Guri, Korea). To analyse hepatic TG and TC contents, livers were homogenized in buffer containing 0.25 M sucrose,

50 mM Tris-HCl and 1 mM EDTA (pH 7.4). Lipids were then extracted from liver homogenates using a chloroform : methanol (2 : 1) mix and quantified as described by Folch *et al.*[16]

Serum alanine aminotransferase activity

Serum alanine aminotransferase (ALT) activity was determined by standard spectrophotometric procedures using a ChemiLab ALT assay kit (IVDLab Co., Uiwang, Korea).

Histological analysis

Oil Red O staining was performed on liver sections. The stained sections were examined using an optical microscope (Olympus Optical Co., Tokyo, Japan). Histological changes were evaluated in randomly chosen histological fields at 200× magnification.

Mitochondrial glutamate dehydrogenase activity

Liver mitochondria were isolated as described by Johnson and Lardy.[17] The isolated mitochondria were homogenized in a solution containing Triton X-100 (0.05% v/v), 50 mM KH_2PO_4 and 1 mM EDTA, pH 7.5 at 4°C. The suspension was used for glutamate dehydrogenase (GDH) assay, according to the method reported by Ellis and Goldberg.[18]

Hepatic lipid peroxidation

Liver homogenates were analysed for malondialdehyde (MDA) by measuring the level of thiobarbituric acid-reactive substance spectrophotometrically at 535 nm with 1,1,3,3-tetraethoxypropane (Sigma-Aldrich, St Louis, MO, USA) as the standard.[19]

Hepatic glutathione content

The total glutathione (GSH) in the liver homogenates was determined spectrophotometrically at a wavelength of 412 nm according to the method reported by Tietze.[20]

Western blot immunoassay

Protein samples (20 μg) from liver homogenates were loaded on polyacrylamide gels, separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and transferred to polyvinylidene difluoride membranes (Millipore, Billerica, MA, USA). The membranes were blocked for 1 h, incubated overnight at 4°C with primary antibodies, and then incubated with the secondary antibodies for 1 h at room temperature. Signal was detected using an enhanced chemiluminescence (ECL) system (iNtRON Biotechnology, Seongnam, Korea), according to the manufacturer's instructions. Intensities of the immunoreactive bands were evaluated densitometrically with the TotalLab TL 120 software (Nonlinear Dynamics Ltd., Newcastle, UK). The following primary antibodies were used: sterol regulatory element-binding transcription factor 1 (SREBP-1) (Abcam, Cambridge, MA, USA; 1 : 2500), carbohydrate-responsive element-binding protein (ChREBP) (Abcam; 1 : 2500), fatty acid synthase (FAS) (Cell Signaling Technology, Beverly, MA, USA; 1 : 2500), phosphorylated-acetyl-CoA carboxylase (phospho-ACC) (Cell Signaling Technology; 1 : 2500), peroxisome proliferator-activated receptor alpha (PPAR-α) (Abcam; 1 : 2500), carnitine palmitoyl-transferase 1A (CPT1A) (Abcam; 1 : 2500), peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1α) (Abcam; 1 : 1000), nuclear respiratory factor 1 (NRF1) (Abcam; 1 : 2000), mitochondrial transcription factor A (TFAM) (Abcam; 1 : 2500), β-actin (Sigma-Aldrich; 1 : 5000) and lamin B1 (Abcam; 1 : 5000).

Statistical analysis

All results are presented as means $\pm$ standard error of the mean. The overall significance of the data was examined by one-way analysis of variance. The differences between the groups were considered significant at $P < 0.05$, with the appropriate Bonferroni correction made for multiple comparisons.

Results

Effect of wild ginseng cambial meristematic cells on body weight, triglyceride and total cholesterol levels, and alanine aminotransferase activity

Animal body weight after consumption of HFD for 10 weeks is presented in Table 2. In the HFD group, body weight significantly increased to 44.6 ± 0.8 g. This increase was attenuated by wild ginseng CMCs 75, 150 and 300 mg/kg. Silymarin 100 mg/kg also attenuated this increase. Hepatic TG and TC levels were 14.2 ± 1.0 mg/g liver and 17.2 ± 1.9 mg/g liver, respectively, in the NFD group. In the HFD group, hepatic TG and TC levels significantly increased to 20.7 ± 1.4 mg/g and 26.8 ± 2.8 mg/g, respectively, which were attenuated by wild ginseng CMCs 75, 150, 300 mg/kg and silymarin 100 mg/kg. In NFD group, the serum TG and TC levels were 83.4 ± 7.7 mg/dl and 141.0 ± 4.0 mg/dl, respectively. The serum TG and TC levels increased in the HFD group to 1.2- and 1.3-fold compared with those in the NFD group. However, wild ginseng CMCs 75, 150 and 300 mg/kg attenuated these increases. Silymarin 100 mg/kg also attenuated these increases. In the HFD group, the level of serum ALT activity increased to 45.5 ± 6.4 U/l, and wild ginseng CMCs 75, 150, 300 mg/kg and silymarin 100 mg/kg showed a tendency to attenuate this increase (Table 2). Based on the results of TG and TC levels and ALT activity, wild ginseng CMCs 150 mg/kg was selected as the optimal effective dose for evaluation of the molecular mechanisms of wild ginseng CMCs against HFD-induced hepatic injury.

Table 2 Effect of wild ginseng CMCs on body weight, lipid metabolism parameters and serum ALT activity in HFD-fed mice

Group	Body weight (g)	Hepatic TG (mg/g liver)	Hepatic TC (mg/g liver)	Serum TG (mg/dl)	Serum TC (mg/dl)	ALT (U/l)
NFD	31.5 ± 0.4	14.2 ± 1.0	17.2 ± 1.9	83.4 ± 7.7	141.0 ± 4.0	36.6 ± 3.9
HFD	$44.6 \pm 0.8^{**}$	$20.7 \pm 1.4^{**}$	$26.8 \pm 2.8^{**}$	$111.2 \pm 5.3^{**}$	$173.2 \pm 9.8^{**}$	45.5 ± 6.4
HFD75	$35.4 \pm 0.7^{**,++}$	$15.1 \pm 1.1^{++}$	$18.6 \pm 1.0^{+}$	$75.2 \pm 5.5^{++}$	156.2 ± 4.3	40.2 ± 7.0
HFD 150	$33.8 \pm 0.5^{++}$	$14.0 \pm 2.1^{+}$	$14.6 \pm 1.7^{++}$	$72.0 \pm 2.8^{++}$	$143.5 \pm 3.3^{+}$	35.8 ± 5.5
HFD 300	$33.2 \pm 0.4^{++}$	$14.8 \pm 1.6^{+}$	$19.8 \pm 1.5^{+}$	$82.6 \pm 5.2^{++}$	150.0 ± 6.2	29.0 ± 4.7
HFD sily	$34.9 \pm 0.1^{**,++}$	$11.7 \pm 1.9^{++}$	$15.4 \pm 0.9^{++}$	$72.8 \pm 4.3^{++}$	$145.2 \pm 5.8^{+}$	31.8 ± 2.5

ALT, alanine aminotransferase; CMCs, cambial meristematic cells; HFD, high-fat diet; NFD, normal-fat diet; sily, silymarin; TC, total cholesterol; TG, triglyceride. All values are means $\pm$ standard error of the mean of 8–10 mice per group. **Significantly different ($P < 0.01$) from the NFD group; [+,++]Significantly different ($P < 0.05$, $P < 0.01$) from the HFD group.
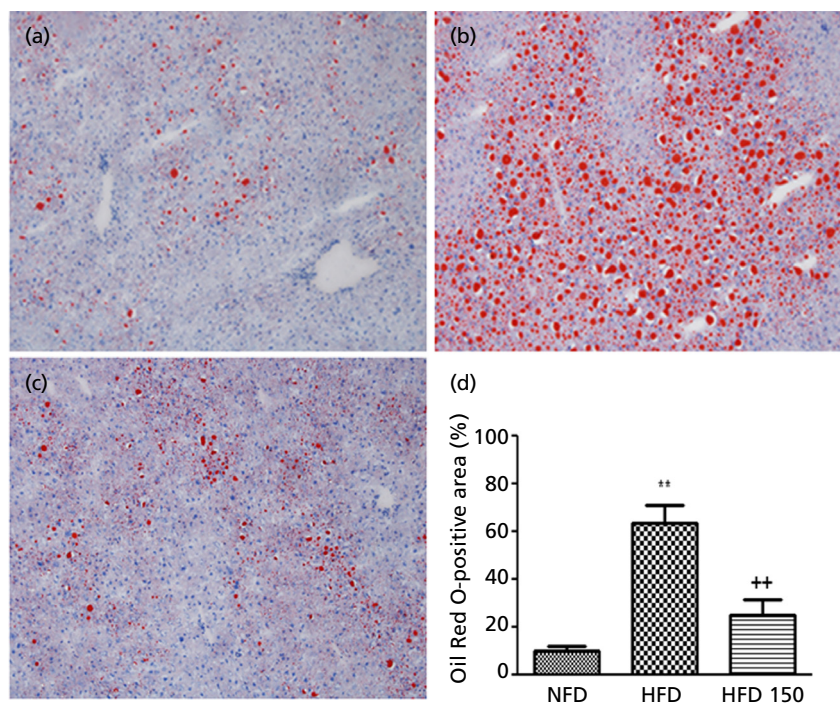
Figure 2 Effect of wild ginseng cambial meristematic cells on liver histology in high-fat diet-fed mice. Histological analysis of steatosis in liver sections stained with Oil Red O (original magnification 200). (a) Liver section from the normal-fat diet group. (b) Liver section from the high-fat diet group. (c) Liver section from the wild ginseng cambial meristematic cells 150 mg/kg-treated high-fat diet group. (d) Quantitative analysis of the Oil Red O staining. **Significantly different ($P < 0.01$) from the normal-fat diet group. ++Significantly different ($P < 0.01$) from the high-fat diet group.

Effect of wild ginseng cambial meristematic cells on histological analysis

The histological features of the NFD group represented normal liver conditions (Figure 2a). HFD-fed mice exhibited a marked increase in lipid accumulation in Oil Red O-stained livers (Figure 2b), and this increase was reduced by wild ginseng CMCs treatment (Figure 2c). Quantitative assessment of fat accumulation in liver indicated that $10.0 \pm 3.7\%$ of liver sections contained fat accumulation (Figure 2d). The Oil Red O-positive area increased to $63.3 \pm 7.6\%$ in livers of HFD group, which was attenuated by wild ginseng CMCs (Figure 2d).

Effect of wild ginseng cambial meristematic cells on the nuclear SREBP-1 and hepatic ChREBP, FAS, P-ACC, PPAR-α and CPT1A protein expression

In the HFD group, the level of nuclear SREBP-1 protein expression significantly increased to 2.0-fold compared with that of the NFD group. This increase was attenuated by wild ginseng CMCs (Figure 3a). The level of hepatic ChREBP protein expression increased in the HFD group to 1.4-fold compared with that of the NFD group. Wild ginseng CMCs attenuated this increase (Figure 3b). As shown in Figure 3c

and 3d, the levels of hepatic FAS and phospho-ACC protein expression increased to 1.5- and 1.3-fold, respectively, compared with those of the NFD group, all of which were attenuated by wild ginseng CMCs. In the HFD group, the levels of hepatic PPAR-α and CPT1A protein expression showed a tendency to increase to 1.6- and 1.3-fold, respectively, compared with those of the NFD group. These increases were enhanced by wild ginseng CMCs (Figure 3e and 3f).

Effect of wild ginseng cambial meristematic cells on hepatic glutamate dehydrogenase activity, malondialdehyde and glutathione level

Hepatic GDH activity was 3.0 ± 0.1 U/mg protein in the NFD group. In the HFD group, GDH level significantly decreased to 1.4 ± 0.1 U/mg protein, and wild ginseng CMCs attenuated this decrease (Figure 4a). In the NFD group, hepatic MDA level was 0.14 ± 0.03 nmol/mg protein. The hepatic MDA level significantly increased in the HFD group to 2.1-fold compared with that in the NFD group; wild ginseng CMCs attenuated this increase (Figure 4b). The level of hepatic GSH was 6.3 ± 0.2 nmol/g liver in the NFD group. In the HFD group, hepatic GSH level significantly decreased to 5.0 ± 0.5 nmol/g liver, which was attenuated by wild ginseng CMCs (Figure 4c).
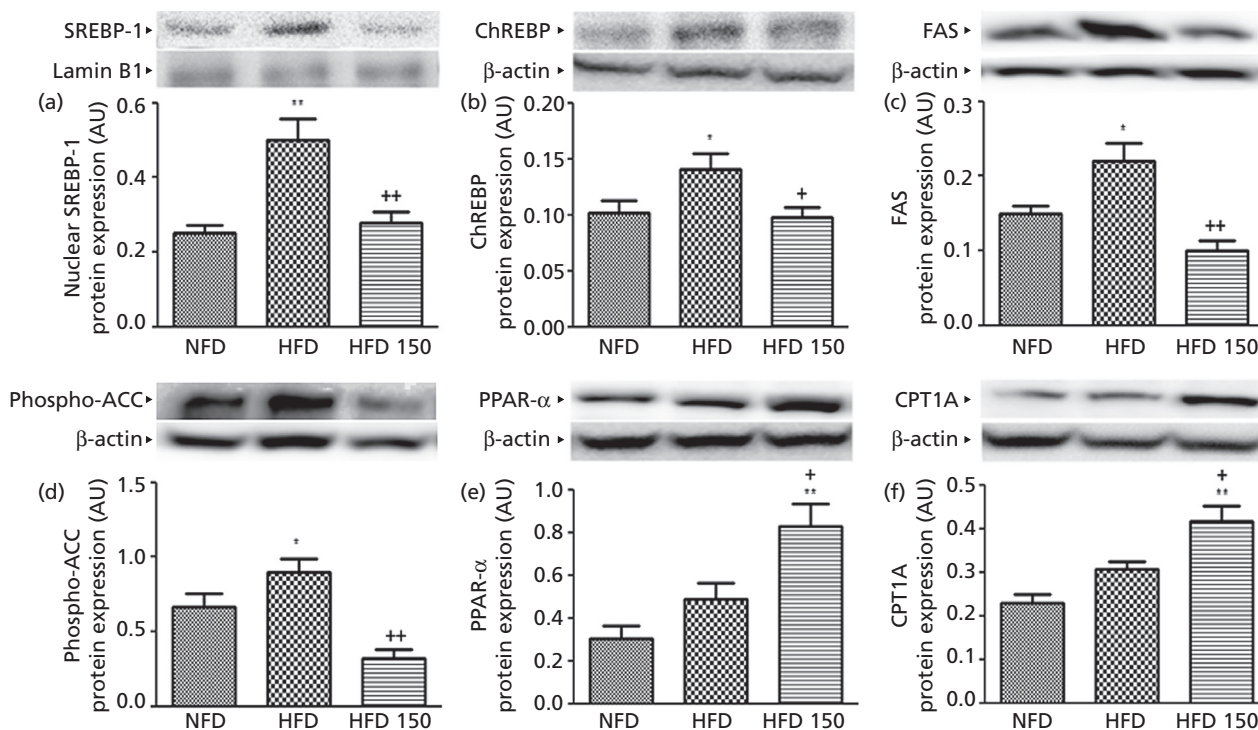
Figure 3 Effect of wild ginseng cambial meristematic cells on nuclear SREBP-1 (a) and hepatic ChREBP (b), FAS (c), phospho-ACC (d), PPAR-α (e) and CPT1A (f) protein expression in high-fat diet-fed mice. All values are means ± standard error of the mean of 8–10 mice group. *,**Significantly different ($P < 0.05$, $P < 0.01$) from the normal-fat diet group. +,++Significantly different ($P < 0.05$, $P < 0.01$) from the high-fat diet group.
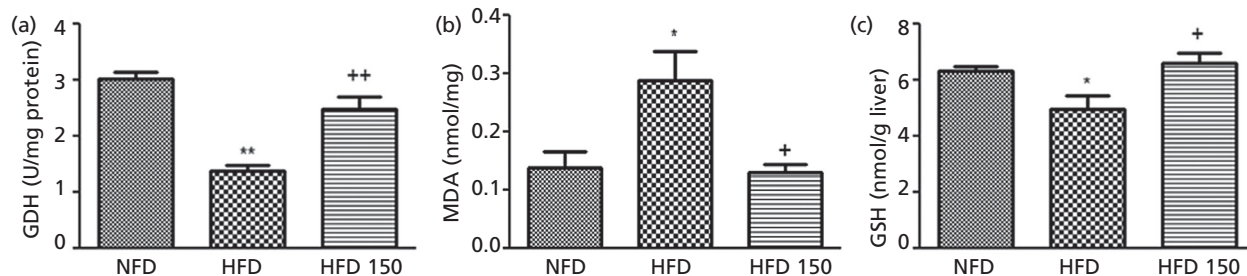

Figure 4 Effect of wild ginseng cambial meristematic cells on hepatic glutamate dehydrogenase activity (a), malondialdehyde level (b) and glutathione content (c) in high-fat diet-fed mice. All values are means + standard error of the mean of 8–10 mice per group. *,**Significantly different ($P < 0.05$, $P < 0.01$) from the normal-fat diet group. +,++Significantly different ($P < 0.05$, $P < 0.01$) from the high-fat diet group.

Effect of wild ginseng cambial meristematic cells on hepatic PGC1α, NRF1 and TFAM protein expression

The administration of HFD did not affect the level of hepatic PGC1 α protein expression. However, wild ginseng CMCs treatment with HFD increased the level of hepatic PGC1α protein expression (Figure 5a). In the HFD group, the hepatic NRF1 and TFAM protein expression increased to 1.5- and 1.4-fold, respectively, compared with those in the NFD group. These increases were enhanced by wild ginseng CMCs (Figure 5b and 5c).

Discussion

Despite extensive research, the pathophysiology of NAFLD remains unclear. Classically, NAFLD has been defined by impairment of fatty acid β-oxidation and excessive de-novo lipogenesis. Recent studies have shown that lipid accumulation in the liver is linked to the progression of endoplasmic
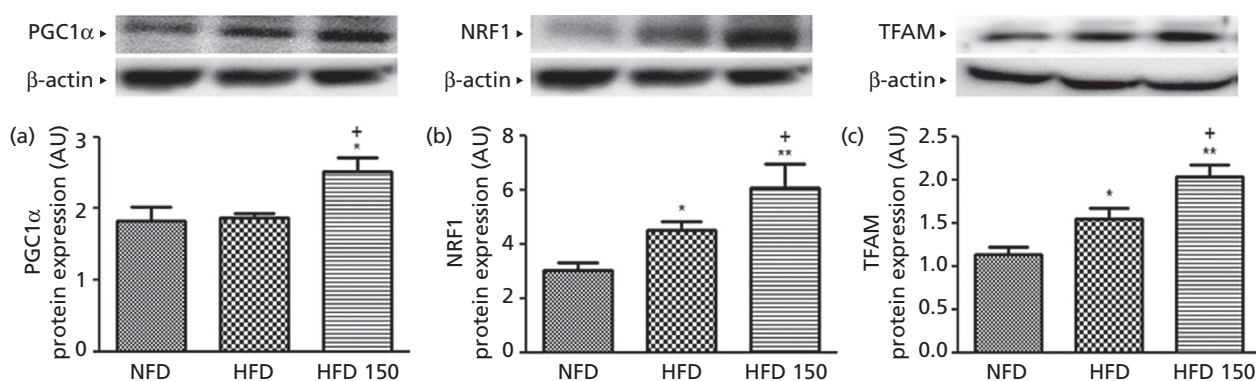
Figure 5 Effect of wild ginseng cambial meristematic cells on hepatic PGC1α (a), NRF1 (b) and TFAM (c) protein expression in high-fat diet-fed mice. All values are means ± standard error of the mean of 8–10 mice per group. *,**Significantly different ($P < 0.05$, $P < 0.01$) from the normal-fat diet group. +Significantly different ($P < 0.05$) from the high-fat diet group.

reticulum stress, mitochondria stress and impaired autophagy.[21] Although the nature of NAFLD has been extensively studied, there is no effective therapy for disease clinically. The use of medicinal plants and their bioactive components continue to be evaluated as potential treatments for NAFLD.[5,22,23] Seo *et al*. reported that *Magnolia officinalis*, a traditional oriental medicine used to treat liver diseases, attenuated the TG synthesis and lipid accumulation in NAFLD.[24] Wild ginseng has also been reported to prevent obesity in animal models by decreasing insulin resistance and body weight.[25] However, there is no information available on the effects of wild ginseng CMCs against hepatic steatosis.

We initially investigated the effect of wild ginseng CMCs on TG and TC levels, which are the representative hallmarks of NAFLD. HFD significantly increased serum and hepatic TG and TC levels. Wild ginseng CMCs attenuated these increases. Furthermore, the increased body weight and ALT level, an indicator of liver damage, in HFD-fed animals were attenuated by wild ginseng CMCs. Histological analyses of liver samples with Oil Red O staining strongly supported the hepatoprotective effect of wild ginseng CMCs. These results indicate that wild ginseng CMCs may potentially have clinical applications in the treatment of fatty liver diseases.

De-novo lipogenesis is the highly regulated metabolic pathway in the liver. Carbohydrates are converted to fatty acids, and these are subsequently esterified to store TG by de-novo lipogenesis. In NAFLD, the balance of de-novo lipogenesis and β-oxidation is collapsed, which subsequently leads to hepatic steatosis. Accumulating evidence shows that increases in both ChREBP and SREBP-1 induced the expression of lipogenic enzymes, including glucokinase, FAS and acetyl CoA carboxylase (ACC).[26] Indeed, knockout of SREBP-1 and ChREBP ameliorated lipid synthesis with decreases in the mRNA levels of fatty

acid synthetic genes.[27,28] Furthermore, ginseng extract inhibited SREBP-1 expression in the liver of HFD-fed mice.[29] In this study, wild ginseng CMCs attenuated the increases in SREBP-1, ChREBP, FAS and phospho-ACC protein expression induced by HFD.

β-Oxidation is the catabolic process through which fatty acid molecules are broken down in the mitochondria.[30] Several medical herbs, such as *Ginkgo biloba*, *Rosa laevigata* and *Zingiber zerumbet*, have been shown to attenuate NAFLD through promotion of fatty acid β-oxidation.[31–33] PPAR-α appears to reflect the sensitivity of induction of lipid oxidation by its ligands, such as fatty acid and eicosanoid.[30] Activation of PPAR-α induces expression of β-oxidation-related genes, including CPT1A, very long chain acyl-CoA dehydrogenases, acyl-CoA oxidase, and liver fatty acid binding protein.[34] Nagasawa *et al*. demonstrated that PPAR-α agonist increased CPT1A mRNA expression and decreased TG level.[34] A previous report suggested that Korean red ginseng increases β-oxidation through PPAR-α-mediated pathways.[35] In our study, PPAR-α and CPT1A protein expression significantly increased in HFD animals, and wild ginseng CMCs enhanced these increases. The increase of PPAR-α observed in our HFD model might be a compensatory effect on HFD-induced liver injury, and wild ginseng CMCs may promote β-oxidation through PPAR-α activation. Collectively, our results suggest that wild ginseng CMCs inhibit de-novo lipogenesis and promote β-oxidation, which prevents lipid accumulation in liver.

Mitochondria are essential compartments in cellular metabolism. Housed within mitochondria are the enzymes of the tricarboxylic acid cycle and β-oxidation, which produce reducing equivalents for the electron transport chain. Hyperlipidemic conditions promote mitochondrial ROS production, which contributes to the oxidative stress developed in NAFLD.[36] Previous studies have reported that *Panax ginseng* extract

decreased lipid peroxidation by activating antioxidant enzymes in the liver from both ageing animal and cadmium-induced hepatotoxicity models.[37,38] Murayama *et al.* evaluated serum hepatic injury markers for the detection of NAFLD and found that GDH, which is specific to mitochondria and located in the matrix, is a more sensitive marker than cytosolic enzymes such as ALT.[39] In this study, HFD-fed mice showed significant decreases in hepatic GDH activity and GSH level, and increase in hepatic MDA level. Wild ginseng CMCs attenuated these changes. Our results suggest that wild ginseng CMCs restore mitochondria dysfunction via suppression of lipid peroxidation in fatty liver disease.

Mitochondrial biogenesis represents an important mechanism by which cells maintain a healthy mitochondrial population.[40] PGC1α is a major regulator of mitochondrial biogenesis activated in response to physiological stimuli, including cold, exercise, fasting and oxidative stress, and controls mitochondrial function through mitochondria remodelling.[41] Activated PGC1α sequentially increases the levels of NRF1 and TFAM. NRF1 functions as a positive regulator of transcription. It acts on genes encoding respiratory chain complex subunits, which are complex I, II, III, IV and V, cytochrome *c* and the transcriptional machinery of the mitochondrion, including TFAM, which is primarily responsible for the transcription and replication of mitochondrial genes from the mitochondrial genome.[42,43] PGC1α knockout model appeared to reduced mitochondrial volume density and respiratory capacity, leading to increased fat mass and weight gain.[44] In the HFD-fed experiment, mitochondrial biogenesis was activated to compensate for mitochondrial dysfunction caused by increased oxidative stress.[45] In our study, PGC1α, NRF1 and TFAM protein levels significantly increased in the liver of HFD-fed animal. Wild ginseng CMCs enhanced the expression of PGC1α, NRF1 and TFAM.

Conclusion

Our findings suggest that wild ginseng CMCs ameliorate HFD-induced hepatic injury by modulating mitochondrial dysfunction and biogenesis. Therefore, wild ginseng CMCs might be useful as a potential therapeutic medication for attenuating NAFLD.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

Funding

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