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The Hypocholesterolemic Effects of *Cistanche tubulosa* Extract, a Chinese Traditional Crude Medicine, in Mice

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Abstract: The roots of *Cistanche* (*C.*) *tubulosa* (Orobanchaceae), a parasitic plant that grows in the Taklamakan desert, are traditionally used as medicines and foods in China. We prepared aqueous ethanol extract (CTE) from the roots of *C. tubulosa* and its hypocholesterolemic effect was evaluated. Using gene chip and RT-PCR analysis of the livers of mice given CTE (400 mg/kg) for 14 days, we found mRNA expression of molecules related to cholesterol transport [apolipoprotein B and very low density lipoprotein (VLDL) receptor] and metabolism [cytochrome P450 side chain cleave (SCC) and steroid 5α-reductase 2] were up-regulated. The administration of CTE (400 mg/kg) for 14 days significantly suppressed serum cholesterol elevation in high cholesterol diet-fed mice. The mRNA expressions of VLDL receptor and cytochrome P450 SCC were significantly enhanced. In addition, acteoside, a major constituent of CTE, was found to enhance the mRNA expressions of apolipoprotein B, VLDL receptor, and cytochrome P450 SCC in HepG2 hepatocytes. These results suggest that CTE affects the mRNA expressions of molecules related to cholesterol transport and metabolism and exhibits hypocholesterolemic activity in diet-induced hypercholesterolemia mice. Acteoside was involved in the hypocholesterolemic activity of CTE.

Keywords: *Cistanche tubulosa*; Cholesterol; Acteoside; Hypercholesterolemia; Apolipoprotein B; Very Low Density Lipoprotein Receptor; Hepatocyte.

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Figure 1. *Cistanche tubulosa.*

Introduction

Cistanche (*C.*) *tubulosa* (Orobanchaceae), a parasitic plant, is cultivated in the Taklamakan desert (Cui *et al.*, 2006, Fig. 1). According to the Chinese Comprehensive Pharmaceutical Dictionary, *C. tubulosa* is used to cure renal disorders, infertility, and menstrual disorders. The plant has been listed in the Chinese pharmacopoeia, and recently, the extract has been prescribed as a Chinese medicine for curing Alzheimer's disease. Besides, *C. salsa,* another wild *C.* species, is widely used clinically as treatment for renal dysfunction and malnutrition. Due to a recent decrease in the yields of crops of *C. salsa*, the application of *C. tubulosa* as an alternative to *C. salsa* has become desirable.

Cistanche species have been reported to display neuroprotective effects in Parkinson's disease (Wang *et al.*, 2007; Geng *et al.*, 2004) and cerebral ischemia-reperfusion (Shi *et al.*, 2004) models. Besides their effects on cerebral function, immunomodulative (Zheng *et al.*, 2002), anti-nociceptive (Lin *et al.*, 2002), anti-inflammatory (Lin *et al.*, 2002), and inhibitory activity against nitric oxide production (Xiong *et al.*, 2000) have also been reported.

C. tubulosa contains various phenylethanoid glycosides (Xie *et al.*, 2006; Yoshikawa *et al.*, 2006). These glycosides have been reported to exhibit neuroprotective effects (Li *et al.*, 2008) and antioxidative activity (Wang *et al.*, 1998). The principal constituents of the glycosides are echinacoside and acteoside (Fig. 2, Chen *et al.*, 2005; Jiang *et al.*, 2009). Echinacoside exhibits neuroprotective activity in striatal dopaminergic neurons in Parkinson's disease model rats (Chen *et al.*, 2007) and mice (Geng *et al.*, 2007). Similarly, acteoside shows a protective effect against neurotoxin-induced apoptosis in cultured cerebellar granule neurons (Pu *et al.*, 2003) and glutamate-induced neurotoxicity in rat cortical cells (Koo *et al.*, 2006). Moreover, acteoside is reported to improve memory in mice (Lee *et al.*, 2006). These reports may support the evidence of *C. tubulosa* for medicinal prescription against Alzheimer's disease. On the other hand, as tissue protective effects, hepatoprotective (Wu *et al.*, 2007; Xiong *et al.*, 1998; Lee *et al.*, 2004), anti-inflammatory (Hausmann *et al.*, 2007), and anti-nephritic (Hayashi *et al.*, 1996) effects have been reported. Thus, echinacoside and acteoside are presumed to repair tissue damage. Regarding their effects on endocrinol and

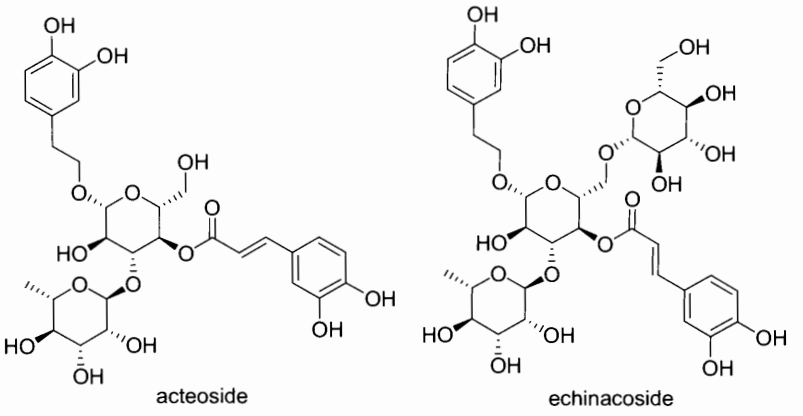

Figure 2. Chemical structures of acteoside and echinacoside.

vascular systems, anti-estrogenic (Papoutsi *et al.*, 2006), angiotensin converting enzyme inhibitory (Kang *et al.*, 2003), vasocontractive (Tam *et al.*, 2002), and hypouricemic (Huang *et al.*, 2008) activities have been reported.

As mentioned above, there are many reports about *C.* species and their phenylethanoid glycosides with regard to cerebral function and inflammation. However, the effects of *C. tubulosa* on lipid metabolism have not been reported. Therefore, we conducted a gene chip analysis of mRNA in the livers of mice given *C. tubulosa* extract (CTE) to find new biological functions for CTE. As a result, CTE was found to affect the mRNA expressions of molecules related to lipid transport and metabolism. Specifically, the mRNA expressions of molecules related to cholesterol were changed. In this paper, we describe the hypocholesterolemic activity of CTE and the changes in the mRNA expression of molecules related to lipid metabolism.

Materials and Methods

Preparation of CTE

CTE (Code No. TLC-34) was manufactured from the dried roots of *C. tubulosa* cultivated in Hotan, Xinjiang, China by Sinphar Pharmaceutical Co. Ltd. (Tung-Shan Shine, Taiwan). The echinacoside and acteoside contents were 25% and 9%, respectively.

Reagents

The GeneChip® Mouse Genome 430 2.0 Array, GeneChip® Expression 3′ Amplification One-Cycle Target Labeling, and control reagents were purchased from Affymetrix (Santa Clara, California, USA). An RNeasy™ Protect Mini Kit was purchased from Qiagen (Hilden, Germany). Random hexamers, 10 mM dNTP mixture, an RNase inhibitor, and fetal calf serum (FCS) were obtained from Invitrogen Co. (Carlsbad, CA, USA).

PrimeScript™ Reverse Transcriptase and SYBR® Green I were purchased from Takara Bio Inc. (Otsu, Japan). Cholesterol, cholic acid and Cholesterol E-Test Wako® were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Dulbecco's modified Eagle medium (D-MEM) and penicillin and streptomycin mixture solution were purchased from Sigma-Aldrich, Inc. (St. Louis, MO, USA).

Animals and Cells

Male ddY mice [5 weeks old and retired mice (approximately: 4 months old)] were obtained from Japan SLC, Inc. (Shizuoka, Japan). The animals were housed in an air-conditioned room (23°C ± 1°C, 50 ± 10% R.H.) for 3 or more days and fed a standard non-purified diet (CE-2, Clea Japan Inc., Shizuoka, Japan) and tap water *ad libitum*. The experiments were performed in accordance with the Guidelines for Animal Experimentation of the Japan Association for Laboratory Animal Science, 1987. HepG2, a human hepatocellular carcinoma cell line (JCRB1054) was obtained from the Health Science Research Resources Bank (Osaka, Japan).

Animal Tests and Determination of Cholesterol

For gene chip analysis and RT-PCR, mice (approximately 4 months old) were orally given CTE (400 mg/kg) once a day for 14 days, and their liver and blood were collected 2 hours after the final administration of CTE. The liver specimens were soaked in RNAlater attached to an RNeasy™ Protect Mini Kit for gene chip analysis and RT-PCR. For evaluation of hypocholesterolemic activity, CTE (200 or 400 mg/kg) was given to the mice (5 weeks old) orally once a day for 14 days. During the breeding period, a high cholesterol diet (HCD) consisting of cholesterol (2%) and cholic acid (0.5%) in a non-purified diet (CRF-1, Oriental Yeast Co. Ltd, Tokyo, Japan) was provided. Two hours after the last administration of CTE, the liver and blood were collected. The serum was separated by centrifugation, and cholesterol was determined using a Cholesterol E-test Wako®. Part of the liver was soaked in RNAlater for RT-PCR analysis. The remaining liver specimens were treated for determination of cholesterol content. Liver specimens (approximately 200 mg) were homogenized in a 19 times weight mixture of chloroform and methanol (2:1) to extract liver lipids. After centrifugation (2800 × g, 10 min), the supernatants (200 μl) were transferred to other test tubes. The solvents were removed by flushing the tube with N_2 gas, and the same volume (200 μl) of phosphate buffered saline without calcium and magnesium [PBS (−)] was added to the tubes. The mixtures were sonicated and their cholesterol contents were determined using the previously mentioned kit.

Gene Chip Analysis of Mouse Livers

One liver specimen each was chosen randomly from the control and CTE groups. Total RNA was extracted from the liver specimen using an RNeasy mini-kit according to the attached protocol. The quantity and quality of each RNA sample was assessed using an

Agilent Bioanalyzer 2100 (Agilent Technologies, Inc. Santa Clara, CA). The preparation of cDNA, cRNA biotinylation and fragmentation, hybridization, and the scanning of probe arrays were performed according to the protocols of the manufacturer (Affymetrix) using the GeneChip® Mouse Genome 430 2.0 Array. The results were analyzed by using Expression Console™ Software (Affymetrix), and the normalization was performed using the MAS5.0 algorithm.

Real Time RT-PCR Analysis of the Mouse Liver

Total RNA in the liver was extracted and purified using the RNeasy™ Protect Mini Kit, and cDNA was synthesized using random hexamers, dNTP mixture, PrimeScript™ Reverse Transcriptase, and an RNase inhibitor. RT-PCR was performed using a Thermal Cycler Dice™ Real Time System (TM800, Takara Bio Inc.), SYBR® Green I, with the following primers. The primers (5′ → 3′) used for the mice RNA experiments were hydroxymethyl-glutaryl (HMG)-CoA reductase (Hmgcr): forward: GCA GAG CCG TCC TCT CTA CAG TA, reverse: ACC TGG TAC TCC TTC CCA TCC AG, apolipoprotein B (Apob): forward: GAA TGA ATG GAG ATG CCA ACC TG, reverse: ACC CAG AGG GAC AAC AAT GGA AT, very low density lipoprotein (VLDL) receptor (Vldlr): forward: GGC CGT CAG CCT GTG ATA CAT AC, reverse: TCA GAG CCA TCA ACA CAG TCT CG, Cytochrome P450 side chain cleave (SCC) (Cyp11a1): forward: CCT GGG TGG CCT ATC ACC AGT AT, reverse: GTC CTG AGC TAC ACC TTC CAG CA, 17′-hydroxylase (Cyp17a1): forward: AGT CTA CCG TTT CTC CCC AGA CG, reverse: CGA CAA GAG GCC TAG AGT CAC CA, 17′-hydroxysteroid dehydrogenase (Hsd17b1): forward: CTT CTG ACC GTT CCC AGA GCT TC, reverse: ACA TCC ACA CGA CCC TCA GTC AC, 3′-hydroxysteroid dehydrogenase (Hsd3b2): forward: ACC TCA CTC CCA CTG TGA TCT GC, reverse: GAC CTT GTC CAG GAC CCT GAT CT, steroid 5′-reductase (Srd5a2): forward: GGT CAT CTA CAG GAT CCC ACA AGG, reverse: ATC CCC AGG AAA CAA AGT GTG AAA, lipin 1 (lipin1): forward: GCT CTT GTC CTC TGC AGT CTT GC, reverse: GGG GCT GGA CTC TTT CAT CTT GT, peroxisome proliferator-activated receptor (PPAR). (Ppara): forward: GAG AGC CCC ATC TGT CCT CTC TC, reverse: GAG CCC GGA CAG CTT CCT AAG TA, carnitie palmitoyltransferase 1 (CPT1): forward: GGA TCT ACA ATT CCC CTC TGC, reverse: GCA AAA TAG GTC TGC CGA CA, β-actin: forward: AAT CGT GCG TGA CAT CAA AG, reverse: GAA AAG AGC CTC AGG GCA T.

Real Time RT-PCR Analysis of HepG2 Hepatocytes

HepG2 cells (4×10^4 cells/500 μl) suspended in D-MEM containing 10% FCS, penicillin (100 μg/ml), and streptomycin (100 μg/ml) were inoculated onto a 24-well culture plate and cultured for 24 hours at 37°C under a 5% CO_2 atmosphere. The medium was changed for a new one containing CTE or its constituents. After 24 hours culture, the medium was removed, and the cells were collected using the solvent included in the RNAeasy™ Protect Mini Kit. Total RNA extraction and cDNA synthesis were performed by a previously described method. The following primers were used for RT-PCR. The primers (5′ → 3′) for human RNA were

apolipoprotein B (Apob): forward: AGC TTC TGG CTT GCT AAC C, reverse: GGC CCC TTG ATA AAC CC, VLDL receptor (Vldlr): forward: GCC AGC AGC TGA AGT CTC TT, reverse: CGG TAA CCA CAT CCA AAG CT, cytochrome P450 SCC (Cyp11a1): forward: TCA CTG CCC CTT TAG CCT GT, reverse: AAT GGC CAT GCT GAA GAT CC, β-actin: forward: CAT CCT CAC CCT GAA GTA CCC CAT CGA G, reverse: ACA GGA CTC CAT GCC CAG GAA GGA AGG C.

Statistics Analysis

The results were expressed as means and SE. The significance of the differences was examined by the student's t-test or one-way ANOVA followed by Dunnett's test. Differences of $p < 0.05$ were considered significant.

Results

Liver mRNA Expressions in Mice

Table 1 shows mRNA expressions related to cholesterol evaluated by gene chip analysis. The mRNA expressions of enzymes related to cholesterol synthesis such as HMG-CoA reductase and mevalonate kinase were suppressed in mice given CTE. With regard to molecules related

Table 1. Liver mRNA Expression of Molecules Related to Cholesterol Biosynthesis and Metabolism in Mice Given CTE

Molecules	Gene Name	Fold Changes vs. Control
Cholesterol Biosynthesis		
HMG-CoA reductase	Hmgcr	0.46
Mevalonate kinase	Mvk	0.46
Mevalonate (diphopho) decarboxylase	Mvd	0.43
Farnesyl diphosphate synthase	Fdps	0.46
Lanosterol synthase	Lss	0.24
Cholesterol Transport and Storage		
Apolipoprotein AI	Apoa1	1.07
Apolipoprotein B	Apob	2.87
Apolipoprotein CII	Apoc2	0.99
LDL receptor	Ldlr	0.85
VLDL receptor	Vldlr	9.00
Lipoprotein lipase	Lpl	2.08
Cholesterol Metabolism		
17β-Hydroxysteroid dehydrogenase	Hsd17b2	0.85
17β-Hydroxysteroid dehydrogenase	Hsd17b4	1.01
17β-Hydroxysteroid dehydrogenase	Hsd17b7	0.65
3β-Hydroxysteroid dehydrogenase	Hsd3b2	1.55
3β-Hydroxysteroid dehydrogenase	Hsd3b3	1.53
3β-Hydroxysteroid dehydrogenase	Hsd3b6	0.65
Aldo-keto-reductase family 1	Akr1d1	2.09
Steroid 5α-reductase 2	Srd5a2	2.23

to cholesterol transport and storage, the mRNA expressions of apolipoprotein B, VLDL receptor, and lipoprotein lipase were obviously enhanced by CTE. 3β-Hydroxysteroid dehydrogenases (Hsd3b2 and Hsd3b3), aldo-keto-reductase family 1, and steroid 5α-reductase 2 are related to cholesterol metabolism. These molecules were enhanced more than 1.5 fold compared to the control.

Besides mRNA expressions related to fatty acid metabolism, lipin 1 and PPARα, which are regulators of fatty acid metabolism, were enhanced in mice given CTE (Table 2). mRNA expressions related to β-oxidation such as that of acetyl CoA acyl transferases (Acaa1a and Acaa1b) and carnitine palmitoyl transferase 1A (Cpt1a) were also up-regulated by CTE.

To confirm the effects of CTE on the mRNA expressions of molecules related to cholesterol, hepatic mRNA expressions were evaluated in mice by RT-PCR. As is shown in Fig. 3, the mRNA expression of HMG-CoA reductase which is a rate-limiting enzyme of cholesterol synthesis was up-regulated by CTE in spite of the result of gene chip analysis. The mRNA expressions of apolipoprotein B and VLDL receptor were significantly enhanced in mice given CTE. With regard to cholesterol metabolizing enzymes, the mRNA expressions of cytochrome P450 SCC and 17α-hydroxylase were significantly enhanced by CTE, in addition to the up-regulation of 17β-hydroxysteroid dehydrogenase and steroid 5α-reductase 2. Besides, 3β-hydroxysteroid dehydrogenase mRNA expression was not enhanced in spite of the gene chip analysis result. With regard to free fatty acid metabolizing enzymes, the mRNA expressions of lipin 1, PPARα, and carnitine palmitoyl transferase 1A were significantly up-regulated by CTE.

Cholesterol Changes and Liver mRNA Expressions in Mice Fed with HCD

Serum cholesterol in mice given CTE (400 mg/kg) was significantly decreased (Table 3). Liver cholesterol was slightly decreased by CTE, however the reduction was not significant.

Table 2. Liver mRNA Expression of Molecules Related to Fatty Acid Biosynthesis and Metabolism in Mice Given CTE

	Gene Name	Fold Changes vs. Control
Fatty Acid Synthesis		
Oleoyl-ACP hydrolase	Olah	1.29
3-Oxoacyl-ACP synthase	Oxsm	0.89
Fatty Acid Metabolism		
Lipin 1	Lpin1	5.11
PPARα	Ppara	2.14
Acetyl CoA acyltransferase 1A	Acaa1a	2.78
Acetyl CoA acyltransferase 1B	Acaa1b	2.07
Carnitine palmitoyltransferase 1A	Cpt1a	2.67
Carnitine palmitoyltransferase 2	Cpt2	0.90
Enoyl-CoA hydratase	Ech	1.21
L-3-Hydroxyacyl-coenzyme A dehydrogenase	Hadhsc	1.15
Hydroxyacyl-coenzyme A dehydrogenase	Hadh	1.21
Acyl CoA oxidase 1	Acox1	1.37
3-Ketoacyl-CoA thiolase	fadA5	0.91

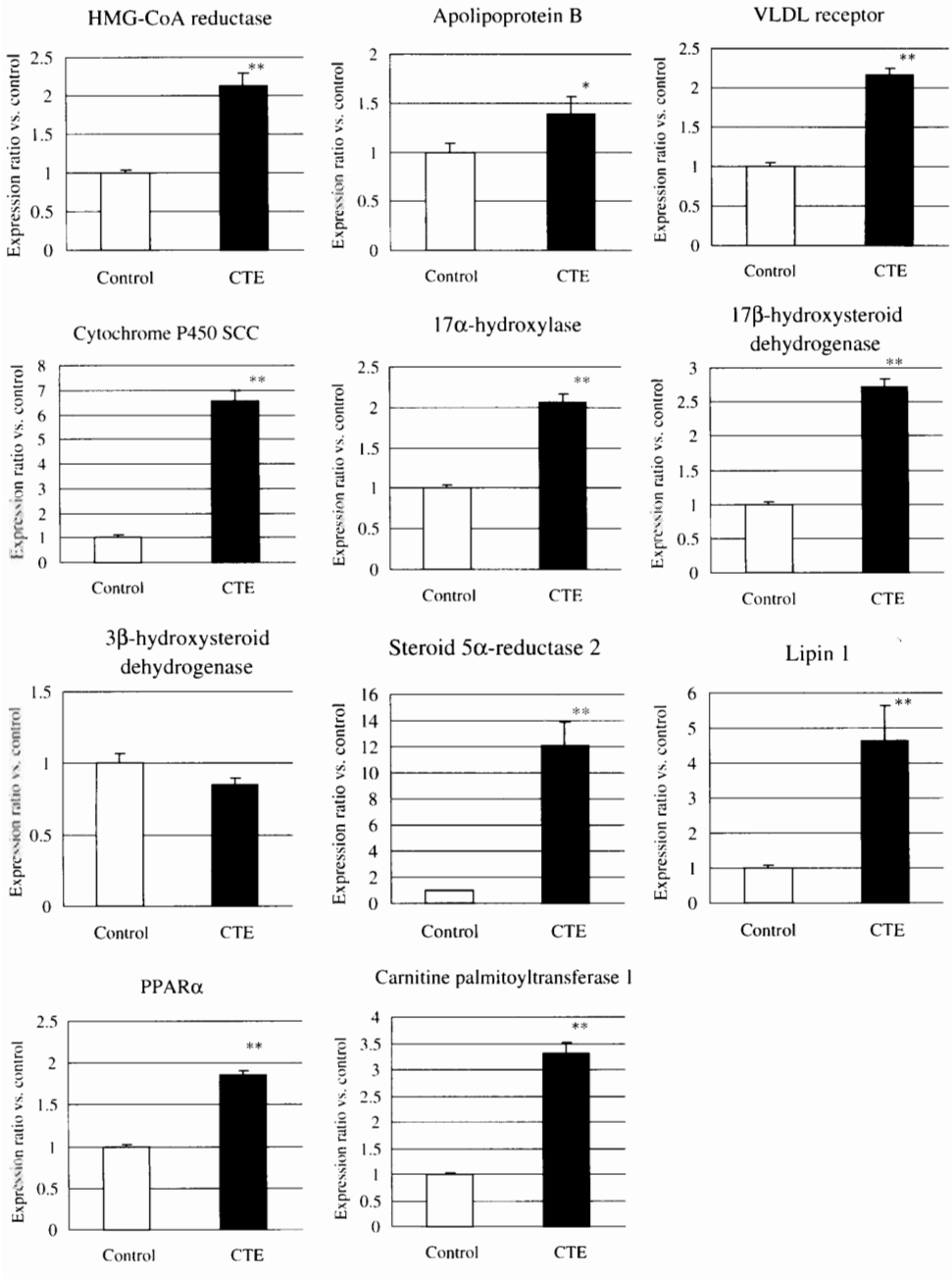

Figure 3. RT-PCR analysis of hepatic mRNA expressions in mice given CTE (400 mg/kg). Each column represents the mean plus SE (n = 7). Asterisks denote significant differences from the control at $^{*}p < 0.05$, $^{**}p < 0.01$, respectively.

Table 3. The Effect of CTE on Serum and Liver Cholesterol in Mice Fed a High Cholesterol Diet

	Dose (mg/kg)	Total Cholesterol	
		Serum (mg/dL)	Liver (mg/g Tissue)
Normal	–	171.3 ± 10.9	16.3 ± 1.9**
Control	–	212.4 ± 25.5	34.3 ± 1.5
CTE	200	185.4 ± 8.0	31.4 ± 2.0
	400	150.6 ± 8.3*	31.8 ± 3.1

Each value represents mean ± SE (n = 7). Asterisks denote significant differences from the control at *p < 0.05, **p < 0.01, respectively.

The mRNA expression of apolipoprotein B tended to be up-regulated by CTE. The mRNA expressions of VLDL receptor, HMG-CoA reductase and cytochrome P450 SCC were significantly enhanced in a dose-dependent manner (Fig. 4).

mRNA Expressions in HepG2 Hepatocytes Treated with CTE and its Constituents

The mRNA expression of apolipoprotein B in HepG2 hepatocytes treated with CTE (30 μg/ml) and acteoside (10 μg/ml) was significantly enhanced (Fig. 5). CTE (3 and 30 μg/ml), and acteoside (10 μg/ml) significantly up-regulated VLDL receptor mRNA expression more than 1.5 fold. Moreover, CTE (30 μg/ml) and acteoside (10 μg/ml) significantly enhanced cytochrome P450 SCC mRNA expression.

Discussion

We utilized gene chip analysis to find novel biological functions for CTE on hepatic lipid metabolism. As a result of CTE administration to the mice, the mRNA expressions of enzymes involved in cholesterol synthesis such as HMG-CoA reductase and mevalonate kinase were suppressed (Table 1). HMG-CoA reductase is a rate-limiting enzyme in cholesterol biosynthesis (Siperstein, 1984), CTE was suggested to suppress cholesterol synthesis in the liver. However, from the result of RT-PCR analysis applied to the other mice, down-regulation of the HMG-CoA reductase mRNA was not detected (Fig. 3). Hence, the down-regulation of the mRNA is suspected to be facultative finding in the mouse. On the other hand, the mRNA expressions of molecules involved in cholesterol transport and metabolism were enhanced. Apolipoprotein B and VLDL receptor act as a cholesterol carrier and a receptor, respectively. The mRNA expression of both molecules was obviously enhanced in livers treated with CTE. Apolipoprotein B is synthesized in the liver and enters the blood stream (Olofsson *et al.*, 2007). It binds with VLDL and LDL and carries them to peripheral tissue. VLDL receptor binds to VLDL and incorporates it into cells (Takahashi *et al.*, 2004) in a similar action to lipoprotein lipase. As CTE enhanced the mRNA expressions of VLDL receptor and lipoprotein lipase, it may enhance cholesterol incorporation

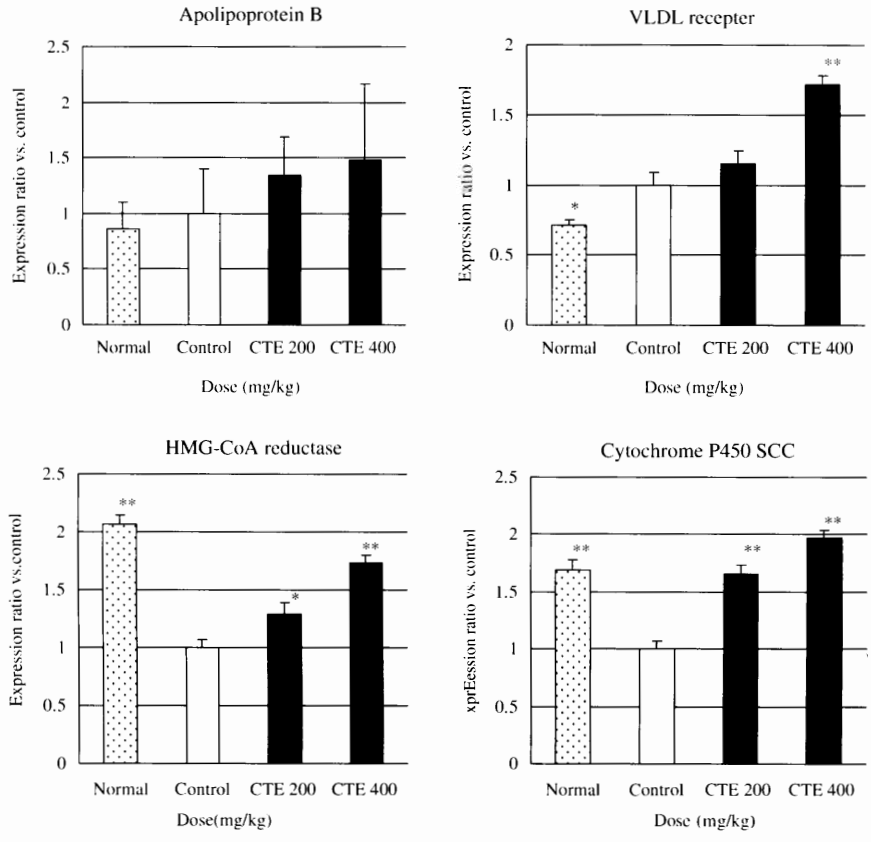

Figure 4. RT-PCR analysis of hepatic mRNA expression in mice given CTE (400 mg/kg) with HCD. Each column represents the mean with plus SE (n = 7). Asterisks denote significant differences from the control at $^{*}p < 0.05$, $^{**}p < 0.01$, respectively.

into hepatocytes. In addition, CTE enhanced the mRNA expression of 3β-hydroxysteroid dehydrogenase (Hsd3b2, Hsd3b3). This enzyme acts in steroid synthesis from cholesterol metabolites. For example, progesterone, androstendione, and testosterone are synthesized from cholesterol metabolites by this enzyme (Payne *et al.*, 2004). 3β-Hydroxysteroid dehydrogenase is reported to exist in the liver as it does in classical steroidogenic tissue (Payne *et al.*, 1997). Moreover, the mRNA expression of aldo-keto-reductase family 1 and steroid 5α-reductase are up-regulated by CTE. These enzymes convert testosterone to dihydrotestosterone, which is a physiologically activated type of testosterone. The enzymes exist in the testes but also in the liver (Jin and Penning, 2001; Jez *et al.*, 1997), and they attenuate the androgen level. From this evidence, CTE is suggested to reduce serum and liver cholesterol levels by enhancing its metabolism. Also, CTE enhanced the mRNA expressions of lipin 1, PPARα, acetyl CoA acyltransferases, and carnitine palmitoyltransferase 1A (Table 2). Lipin 1 (Finck *et al.*, 2006) and PPARα (Hashimoto *et al.*, 1999) are regulators of lipid metabolism in the liver. Acetyl CoA acyltransferases are involved in fatty acid β-oxidation in the liver

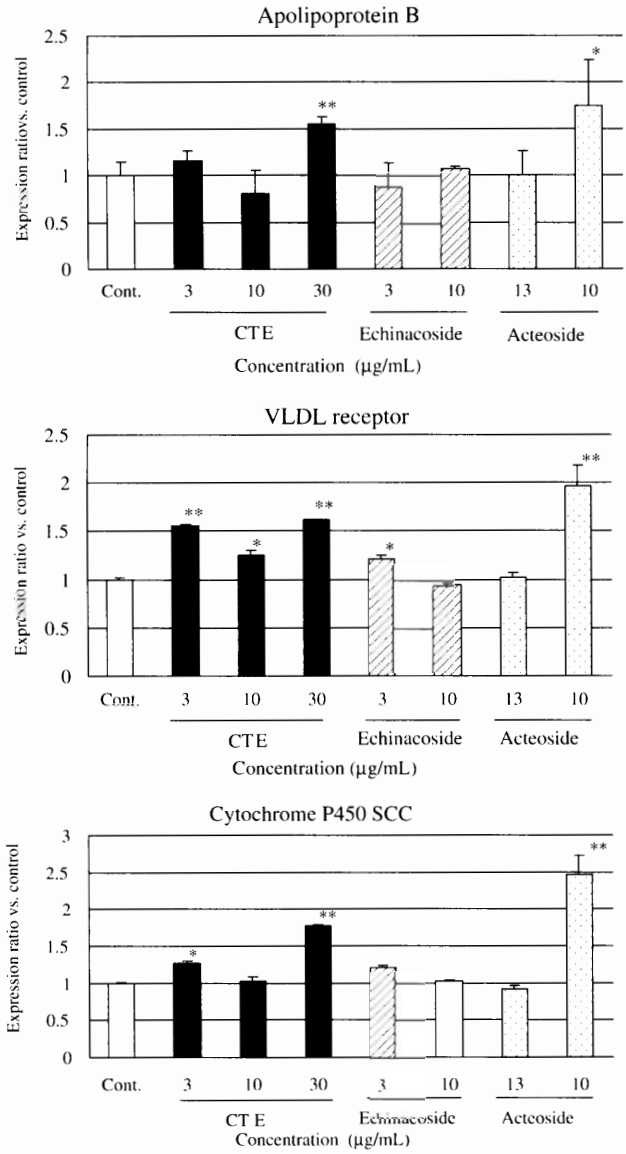

Figure 5. The effect of CTE on the mRNA expression of apolipoprotein B, VLDL receptor and cytochrome P450 SCC in HepG2 cells. Each column represents the mean plus SE (n = 4). Asterisks denote significant differences from the control at $^{*}p < 0.05$, $^{**}p < 0.01$, respectively.

(Fujiki *et al.*, 1985) and carnitine palmitoyltransferase 1A is involved in the up-take of fatty acids into mitochondria for β-oxidation (López-Viñas *et al.*, 2007). Thus, it is possible that CTE enhances free fatty acid metabolism in the liver.

To confirm the effects of CTE obtained from the gene chip analysis, RT-PCR analysis was applied to measure several mRNAs in livers obtained from other mice. The mRNA

expressions of apolipoprotein B, VLDL receptor, 17β-hydroxysteroid dehydrogenase, and steroid 5α-reductase 2 were confirmed to be enhanced by CTE (400 mg/kg, Fig. 3). The mRNA expressions of lipin 1, PPARα, and carnitine palmitoyltransferase 1 were also significantly enhanced. In addition to the above evaluation, we assessed the other mRNA expressions of enzymes related to cholesterol metabolism. The mRNA expressions of cytochrome P450 SCC and 17α-hydroxylase, which are related to cholesterol metabolism, were significantly up-regulated. However, the mRNA expression of 3β-hydroxysteroid dehydrogenase was not affected by CTE. From the result of the RT-PCR, CTE was found to generally affect the mRNA expressions of enzymes involved in cholesterol transport and metabolism. Moreover, CTE may enhance free fatty acid β-oxidation in liver, because CTE enhanced the mRNA expressions of lipin 1 and carnitine palmitoyltransferase 1.

We continuously examined whether CTE suppresses serum and liver cholesterol in diet-induced hypercholesterolemia model in mice. As a result, CTE was found to suppress serum cholesterol level (Table 3). The mRNA expressions of apolipoprotein B, VLDL receptor, and cytochrome P450 SCC were enhanced and that of HMG-CoA reductase was also up-regulated. The up-regulation of HMG-CoA reductase by CTE, which was also detected in normal mice, is not seen to affect serum and liver cholesterol elevations. Hence, CTE is suggested to exhibit a hypocholesterolemic effect in diet-induced hypercholesterolemia mice by enhancement of cholesterol transport and metabolism in liver.

To clarify the involvement of echinacoside and acteoside, which are major constituents of CTE, on the hypocholesterolemic activity of CTE, we evaluated their effect on the mRNA expressions of apolipoprotein B, VLDL receptor, and cytochrome P450 SCC in HepG2 hepatocytes. CTE (30 μg/ml) and acteoside (10 μg/ml) enhanced the mRNA expression of all molecules. However, echinacoside (10 μg/ml) did not enhance their mRNA expressions. Thus, acteoside was found to be involved in the hypocholesterolemic effect of CTE.

In conclusion, we found hypocholesterolemic activity in *C. tubulosa* extract. The mechanism was presumed to be based on the enhancement of cholesterol transport into the liver and its hepatic metabolism. Acteoside, a major constituent of *C. tubulosa* extract may be responsible for the hypocholesterolemic activity of CTE.

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