Protective Effects of *Cinnamomum cassia* Blume in the Fibrogenesis of Activated HSC-T6 Cells and DimethylNitrosamine-Induced Acute Liver Injury in SD Rats

Chang-Shin Lim,¹ Eun-Young Kim,¹ Hyun-Sam Lee,¹ Yunjo Soh,² Youngjoo Sohn,³ Sun Yeou Kim,⁴ Nak-Won Sohn,⁴ Hyuk-Sang Jung,¹,⁴ and Yoon-Bum Kim¹,²

¹College of Oriental Medicine and Institute of Oriental Medicine, Kyung Hee University, 1, Hoegi-dong, Dongdaemun-gu, Seoul, 130-701, Republic of Korea
²Department of Dental Pharmacology, School of Dentistry, Chonbuk National University, Jeonju, 561-756, Republic of Korea
³College of Oriental Medicine, Sangji University, 660, Usan-dong, Wonju-Si, Gangwondo, 220-702, Republic of Korea
⁴Graduate School of East-West Medicine, Kyung Hee University, 1, Seocheon-dong, Kihung-ku, Youngin-City, Kyungki-Do, 449-701, Republic of Korea

Received June 19, 2009; Accepted December 5, 2009; Online Publication, March 7, 2010 [doi:10.1271/bbb.90435]

*Cinnamomum cassia* Blume (CC) is one of the world’s oldest natural spices, and is commonly used in traditional oriental medicine. We investigated the protective effect of ethanol extract from *Cinnamomum cassia* Blume (CCE) on the activation of hepatic stellate cells (HSCs). In addition, we examined the effects of CC powder in Sprague-Dawley rats with acute liver injury induced by dimethylnitrosamine (DMN). In vitro, HSC-T6 cells exhibit an activated phenotype, as reflected in their fibroblast-like morphology. CCE significantly reduced the expression of alpha-smooth muscle actin (α-SMA), connective tissue growth factor (CTGF), transforming growth factor beta (TGF-β), and tissue inhibitor of metalloproteinase-1 (TIMP-1), and tissue inhibitor of metalloproteinase-1 (TIMP-1). In vivo, the results were significantly protected by CC powder in the serum total protein, albumin, total-bilirubin, direct-bilirubin, glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), and alkaline phosphatase (ALP). We suggest that CC inhibits fibrogenesis, followed by HSC-T6 cell activation and increased restoration of liver function, ultimately resulting in acute liver injury.

Key words: hepatic stellate cell (HSC)-T6; alpha-smooth muscle actin (α-SMA); dimethylnitrosamine; *Cinnamomum cassia* Blume

Hepatic fibrosis is a wound-healing response to chronic liver injury that can lead to cirrhosis and liver failure if repetitive liver damage occurs. Currently, fibrosis is known to be part of a dynamic process of continuous extracellular matrix (ECM) remodeling in the setting of chronic liver injury, which leads to an excessive accumulation of several extracellular proteins, proteoglycans, and carbohydrates.¹ ² The damaged hepatocytes, their membrane components, metabolites of toxic agents, and infiltrating inflammatory cells are activators of Kuffer cells.³ Activated Kuffer cells release a number of soluble agents, including cytokines, reactive oxygen species, and other factors. These factors act as activators of hepatic stellate cells (HSCs).⁴ HSCs have been established as the primary source of ECM in liver injury.⁵ In the normal liver, HSCs comprise approximately 1.4% of total liver volume and are present at a ratio of about 3.6 to 6 cells per 100 hepatocytes.⁶ After acute liver injury and in chronic liver disease, quiescent HSCs become activated and trans-differentiate into myofibroblast-like cells characterized by several key phenotypic changes, such as enhanced cell migration and adhesion, expression of alpha smooth muscle actin (α-SMA), increased proliferation, accumulation of ECM, including α1(I) collagen, and loss of stored vitamin A droplets.⁷ ⁸

DimethylNitrosamine (DMN) is a potent hepatotoxin, carcinogen, and mutagen. It is well known that repeated injections of low-dose DMN cause liver fibrosis or cirrhosis.⁹ A low dose of DMN initially causes diffuse hemorrhagic necrosis, leading to mononuclear cell infiltration of the liver⁹ ¹⁰ and subacute and chronic liver injury, with varying degrees of fibrosis and nodular regeneration.¹¹ ¹² A high dose of DMN administered in one injection to an experimental animal resulted in submassive necrosis that resembled human fulminating hepatitis.¹³ Hence this compound has been widely used as a model agent to study the mechanisms of liver injury.

*Cinnamomum cassia* Blume (CC) is one of the world’s oldest natural spices, and is a commonly used...
herb in traditional oriental medicine. In traditional oriental medicine, CC is a medicinal plant that is used to treat dyspepsia, gastritis, blood stasis, and inflammatory diseases. It is reported to reduce blood pressure in experimental rats, and it has significant antiallergic, antifulgogenic, antipyretic, and anaesthetic effects. It has been studied recently in diabetics for its anti-diabetic effects. It also has various effects in the digestive system, but the effects of CC on liver damage have not been studied extensively. In this present study, the effects of an ethanol extract of *Cinnamomum cassia* Blume (CCE) on the index of liver fibrosis were investigated in activated HSC-T6 cells. In addition, we examined the effects of CC powder in Sprague-Dawley rats with acute liver injury induced by DMN.

**Materials and Methods**

*HPLC analysis of standard material for sample testing.* Extraction of cinnamaldehyde from CC hot water extract was carried out according to the modification of the method of He et al. About 2 g of a mixed, fine-powdered sample of CC was weighed, put in a 500-mL round plate bottom flask, dissolved with 50 mL of methanol (HPLC grade, 4 liters, Millinckrodt Baker, Phillipsburg, NJ) by refluxing for 2 h at 75 °C twice, and then centrifuged for 15 min at 3000 rpm at room temperature (RT). The upper solution was filled to 200 mL with methanol. The standard material was accurately weighed to 10.0 mg and dissolved in 10 mL of methanol. The dissolved standard solution was diluted to 0.25, 0.5, and 1.0 mg/mL. Afterwards, a standard HPLC chromatogram was obtained.

HPLC analysis was carried out with a Waters 2695 Alliance System with a 2996 PDAD detector and a Waters Empower system (Ver. 5.00, Waters, Milford, MA). The test sample was analyzed by reverse-phased HPLC on a C18 column (X TerraMS, 5 μm ODS, 150 mm × 3.9 mm (L,D), Waters) in an isocratic condition of acetonitrile/water/acetic acid glacial (30:69:1 v/v) at 0.8 mL/min with detection at 280 nm and injections in portions of 10 μL; the total run time was 30 min.

**Effects of CC on HSC-T6 cells.**

**Preparation of CCE.** One-hundred g of CC was extracted with 80% ethanol (RT) for 2 weeks and filtered with He et al. About 2 g of a mixed, fine-powdered sample of CC was weighed, put in a 500-mL round plate bottom flask, dissolved with 50 mL of methanol (HPLC grade, 4 liters, Millinckrodt Baker, Phillipsburg, NJ) by refluxing for 2 h at 75 °C twice, and then centrifuged for 15 min at 3,000 rpm at room temperature (RT). The upper solution was filled to 200 mL with methanol. The standard material was accurately weighed to 10.0 mg and dissolved in 10 mL of methanol. The dissolved standard solution was diluted to 0.25, 0.5, and 1.0 mg/mL. Afterwards, a standard HPLC chromatogram was obtained.

**HPLC analysis** was carried out with a Waters 2695 Alliance System with a 2996 PDAD detector and a Waters Empower system (Ver. 5.00, Waters, Milford, MA). The test sample was analyzed by reverse-phased HPLC on a C18 column (X TerraMS, 5 μm ODS, 150 mm × 3.9 mm (L,D), Waters) in an isocratic condition of acetonitrile/water/acetic acid glacial (30:69:1 v/v) at 0.8 mL/min with detection at 280 nm and injections in portions of 10 μL; the total run time was 30 min.

**Effects of CCE on HSC-T6 cells.**

**Preparation of CCE.** One-hundred g of CC was extracted with 80% ethanol (RT) for 2 weeks and filtered with He et al. About 2 g of a mixed, fine-powdered sample of CC was weighed, put in a 500-mL round plate bottom flask, dissolved with 50 mL of methanol (HPLC grade, 4 liters, Millinckrodt Baker, Phillipsburg, NJ) by refluxing for 2 h at 75 °C twice, and then centrifuged for 15 min at 3,000 rpm at room temperature (RT). The upper solution was filled to 200 mL with methanol. The standard material was accurately weighed to 10.0 mg and dissolved in 10 mL of methanol. The dissolved standard solution was diluted to 0.25, 0.5, and 1.0 mg/mL. Afterwards, a standard HPLC chromatogram was obtained.

HPLC analysis was carried out with a Waters 2695 Alliance System with a 2996 PDAD detector and a Waters Empower system (Ver. 5.00, Waters, Milford, MA). The test sample was analyzed by reverse-phased HPLC on a C18 column (X TerraMS, 5 μm ODS, 150 mm × 3.9 mm (L,D), Waters) in an isocratic condition of acetonitrile/water/acetic acid glacial (30:69:1 v/v) at 0.8 mL/min with detection at 280 nm and injections in portions of 10 μL; the total run time was 30 min.

**Western blot analysis for α-SMA, CTGF, PCNA, PDGFR-β, and TGF-β1.** HSC-T6 cells (5 × 10^4) were seeded on a P-100 dish (Corning, NY) in DMEM supplemented with 10% FBS. After 24 h, the HSC-T6 cells were exposed to various concentrations of CCE (1–100 μg/mL) in the presence of 100 μM PMA for 24 h. The cells were washed twice with cold phosphate-buffered saline (PBS) and lysed with cell lysis buffer (40 mM Tris–HCl, 10 mM EDTA, 120 mM NaCl, 1% Nonidet P-40, 1 mM PMSF, 1 mM Na3VO4, 1% Triton X-100). The cell lysates were centrifuged at 13,000 rpm for 20 min. The supernatants were collected and stored at −70 °C until detection. Protein concentrations were analyzed with a Bio-Rad protein assay kit (Bio-Rad, Hercules, CA). Proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and electrotransferred onto a PVDF membrane (Millipore, Bedford, MA). The membranes were blocked with 5% non-fat milk for 1 h and incubated with anti-TGF-β1 antibody (1:1,000 dilution, Promega), anti-α-SMA antibody (1:1,000 dilution, Neomarker), anti-PCNA (1:1,000 dilution, Santa Cruz), anti-CTGF (1:1,000 dilution, Santa Cruz), anti-actin (1:1,000 dilution, Santa Cruz), and anti-PDGFR-β (1:1,000 dilution, Santa Cruz) overnight at 4 °C, and then incubated with a suitable horseradish peroxidase (HRP)-conjugated IgG antibody for 1 h at RT.

**Western blot analysis for TIMP-1.** HSC-T6 cells (2 × 10^5) were seeded on a 6-well plate (Corning, NY) in DMEM supplemented with 10% FBS. After 24 h in DMEM supplemented with 10% FBS, the medium was replaced with serum-free DMEM with various concentrations of CCE (1, 10, and 20 μg/mL) in the presence of 100 nM PMA. After 24 h, the medium was harvested, briefly centrifuged, and immediately frozen at −70 °C. Aliquots from identical numbers of HSC-T6 cells were separated by SDS-PAGE and electrotransferred onto a PVDF membrane (Millipore). The membranes were blocked with 5% non-fat milk for 1 h and incubated with anti-TIMP-1 antibody (1:1,000 dilution, Santa Cruz) overnight at 4 °C, and then incubated with horseradish peroxidase (HRP)-conjugated IgG antibody for 1 h at RT, and the antibody specific proteins were visualized by an enhanced chemiluminescence (ECL) procedure using an ECL detection reagent (Amersham Pharmacia, Piscataway, NJ). Each immunoreactive band was quantitated with Scion Image Software.

**Effects of CC on DMN-induced acute liver injury in rats.**

**Preparation of animals.** Animal care and all experimental procedures were conducted in accordance with the “Guide for Animal Experiments” edited by the Korean Academy of Medical Sciences. Six-week-old male Sprague-Dawley rats (Corning, NY) were purchased from Samtako (Seoul, Korea). The rats were housed at 22 ± 2 °C at 50 ± 10% humidity under a 12-h-12 h light-dark cycle. The rats were given autoclaved diets (Lab Diet #5001, PMI Nutrition International, St. Louis, MO) and water. Prior to the experiments, the rats were allowed to adapt to the laboratory environment for 1 week. DMN was purchased from Sigma (St. Louis, MO). Silymarin, named Legalon 70 (Bukwang Pharmaceuticals, Seoul, Korea) was purchased from a local drugstore. CC was from Omniherb (Youngcheon, Korea).

**Induction of acute liver injury.** The 32 rats were divided into four groups of eight rats each. The normal group was treated with intraperitoneal injection of 0.15 M NaCl. The control (−) group was treated with an intraperitoneal injection (0.1 mL/100 g) of DMN (diluted with 0.15 M NaCl) at a dose of 40 mg/kg. The silymarin group was treated with an intraperitoneal injection of DMN at a dose of 40 mg/kg, followed by daily treatment with silymarin (suspended in 1 mL of D.W.) at a dose of 100 mg/kg by oral gavage for 4 days. The CC group was given an intraperitoneal injection of DMN (diluted with saline) at a dose of 40 mg/kg, followed by daily treatment with CC powder (suspended in 1 mL of D.W.) at a dose of 100 mg/kg/100 g by oral gavage for 4 days. At the end of the study, all the rats in each group were sacrificed under anesthesia with diethyl ether. The liver and spleen were removed immediately and weighed.

**Biochemical analysis of serum.** Blood samples for biochemical analysis were obtained from the left ventricle. Serum total protein (T-protein), albumin, total bilirubin (T-Bili), direct bilirubin (D-Bili), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), and alkaline phosphatase (ALP) were entrusted to SCL (Seoul Clinical Laboratories, Seoul, Korea).

**Results and Discussion.**

**1. Biochemical analysis of serum.**

**2. Histological analysis of liver.**

**3. Western blot analysis for α-SMA, CTGF, PCNA, PDGFR-β, and TGF-β1.**

**4. Western blot analysis for TIMP-1.**
Measurement of hepatic hydroxyproline assay. The hydroxyproline content was determined by the Jamall method, as previously reported. Liver tissue (0.3 g) was homogenized in 6 N HCl and hydrolyzed at 110 °C for 18 h. Twenty-five microliter aliquots were dried at 60 °C. The sediment was dissolved in 1.2 ml of 50% isopropanol and incubated with 200 ul of 0.56% chloramine T Solution (Sigma) in acetate citrate buffer pH 6.0. After incubation for 10 min at RT, 1 ml of Ehrlich’s reagent was added, and the mixture was incubated at 50 °C for 90 min. After cooling, absorbance was measured at 558 nm (BECKMAN, Fullerton, CA, DU530).

Liver histopathology. The liver specimens were fixed in 10% neutral buffered formalin and embedded in paraffin. Four-micrometer liver sections were deparaffinized and processed routinely for hematoxylin-eosin (H-E), Masson trichrome, and Van Gieson staining.

Immunohistochemical study. Endogenous peroxidases of the samples for α-SMA were blocked by incubation in 3% hydrogen peroxide in methanol for 30 min. The samples were washed 3 times with tris-buffered saline (TBS, pH 7.4) and blocked for 10 min at RT with 10% normal horse serum (Vector, Burlingame, CA) in TBS. After they were washed 3 times with TBS, the samples were incubated for 18 h at 4 °C with the appropriate dilution of primary antibody. We used antibodies against mouse α-SMA diluted 1 : 200 (Neomarker, Fremont, CA). The slides were rinsed with TBS, incubated for 30 min with vectastain ABC solution (enzyme-labeled complexes, Vector, Burlingame, CA), and washed with TBS, after which the signal was detected using 3,3-diaminobenzidine (DAB; Sigma). Alpha-SMA were counterstained with hematoxylin and eosin respectively, and mounted in Canada balsam. The stained sections were examined using an optical microscope (Olympus, Tokyo, BX51).

Imaging analysis. For morphometric analysis, we assessed the mean value of the area of α-SMA positive cells in three ocular fields per specimen as percent area at 40× magnification using an image-analysis system (NIH image 1.62, Bethesda, MD). Alpha-SMA positive cells were expressed as a percentage of the total area of the specimen.

Statistical analysis. All values were expressed as the mean ± standard error. Raw data were subjected to one-way ANOVA, followed by Dunnett’s post-hoc comparisons. A p-value of ≤0.05 was considered statistically significant.

Results

HPLC analysis of standard material for sample testing
From the results for the standard calibration curve, the amounts (mg) of the two standard materials in CC methanol extracts were calculated from the relationship between the concentration and peak area, which were obtained using known amounts of trans-cinnamic acid
and cinnamaldehyde. The amounts of trans-cinnamic acid and cinnamaldehyde in the CC methanol extracts were qualified as $4.54 \pm 0.20$ mg/g of extracts (0.45 ± 0.02%) and $141.90 \pm 4.49$ mg/g of extracts (14.19 ± 0.45%) respectively (Fig. 1).

**Effects of CCE exposure on HSC-T6 cell viability**

The response of HSC-T6 cell lines following exposure to CCE is illustrated in Fig. 2. One, 10, and 100 µg/ml of CCE treatment did not affect viability under the serum condition (Fig. 2). The cellular viabilities were estimated to be greater than 90%. Each data point represents the average of a series of three different experiments.

**Changes in the expression of α-SMA, CTGF, PCNA, PDGFR-β, and TGF-β1**

To determine the effects of CCE on α-SMA, CTGF, PDGFR-β, TGF-β1, and PCNA, Western blotting analysis was applied to detect the protein levels. The protein levels of α-SMA and PDGFR-β in CCE-treated HSC-T6 decreased gradually with increases in the extract concentration from 1 to 100 µg/ml (Fig. 3). Compared with the untreated normal group, the production of TGF-β1 and CTGF was not affected by treatment with 1 and 10 µg/ml CCE. However, 100 µg/ml of CCE decreased the protein levels of TGF-β1 and CTGF by about 31.3% and 81.8% respectively. The protein level of PCNA was not different between the CCE-treated group and the normal group.

**Change in the expression of TIMP-1**

We examined the effect of CCE on the level of TIMP-1 in HSC-T6 cells induced by PMA. Western blotting was performed to determine the effects of CCE on TIMP-1 protein release in the culture medium. As shown in Fig. 4, PMA induced the expression of TIMP-
1 in the HSC-T6 cells. Compared with the PMA treated group, 1, 10, and 20 μg/ml of CCE reduced the expression of TIMP-1 by about 14.5%, 25.8%, and 64.7% respectively after the 24-h treatment (Fig. 4).

Biochemical analysis of serum

The effects of CC powder on serum parameters in the acute liver injury model are shown in Fig. 5. Serum T-protein \((p < 0.001)\), albumin \((p < 0.001)\), T-Bili \((p < 0.01)\), D-Bili \((p < 0.01)\), GOT \((p < 0.01)\), GPT \((p < 0.05)\), and ALP \((p < 0.001)\) significantly recovered in the CC-treated group as against control (-). On the other hand, the silymarin-treated group significantly decreased only in ALP \((p < 0.001)\) (Fig. 5).

Change in \(\alpha\)-SMA expression

Expression of \(\alpha\)-SMA, an indicator of activated HSCs, was detected by the immunohistochemistry method. The means of the \(\alpha\)-SMA positive regions at 100× magnification were 1.89 ± 0.21%, 1.65 ± 0.22%, and 1.44 ± 0.15% in the control (--), silymarin-, and CC-treated group respectively at 4 after DMN injection. Activation of \(\alpha\)-SMA was inhibited in the CC-treated group (Fig. 6).

Discussion

The HSC-T6 cell line exhibits an activated phenotype and displays a fibroblast-like morphology. The HSC-T6 cell line is constructed by transfection with SV40 sequences into rat HSCs, and its phenotype is activated HSCs. The infiltrating cells trigger activation of HSC-secreted cytokines such as TGF-\(\beta\) and PDGF. Typically, liver injury leads to activation of HSCs, and progresses to chronic fibrosis and finally to cirrhosis with chronic liver failure. Hence it is important that medicinal treatment of liver injury should include not only restoration of liver function but also inhibition of liver fibrosis.

Among the many drugs for liver injury, silymarin is the clinically most prescribed drug. It is known to have hepatotherapeutic and anti-fibrotic properties. Hence we used silymarin as the positive control in this experiment.

In hepatic fibrosis, an increase in TGF-\(\beta\) has been demonstrated in cirrhosis, experimental hepatic fibrosis, and cultured activated HSCs. TGF-\(\beta\) leads to perpetual HSC activation by prompting HSCs to transit into myofibroblasts, stimulating the synthesis of ECM (extracellular matrix) and inhibiting its degradation. TGF induces overexpression of CTGF during hepatic fibrogenesis. CTGF is selectively induced by TGF-\(\beta\) in fibroblastic cell types, and it plays a key role in the
overproduction of ECM in activated HSCs. CTGF expression in HSCs is significantly enhanced during the activation process in vitro and in vivo.\(^{31,32}\)

Our experiment indicated that TGF-\(\beta\)1 and CTGF were decreased by CCE treatment. Considering that CCE decreased TGF-\(\beta\)1 and CTGF expression at the same concentration, CCE might control CTGF through inhibition of TGF-\(\beta\)1. Expression of PDGFR is also one of the key events in HSC activation. PDGFR participates in proliferation and migration response.\(^{33,34}\) PDGF is the most potent proliferative stimulus towards stellate cells.\(^{34}\) Both PDGF\(^{35}\) and its receptor\(^{36}\) are upregulated following liver injury. The mitogenic capability of PDGF in stellate cells requires activation-dependent expression of the receptor.\(^{37}\) Quiescent stellate cells express PDGFR-\(\alpha\), while activated stellate cells express both PDGFR-\(\alpha\) and PDGFR-\(\beta\).\(^{36}\)

Myofibroblasts that synthesize and secrete collagen I during wound healing are characterized by the expression of \(\alpha\)-SMA.\(^{30}\) Activated HSC is associated with cell proliferation and the accumulation of ECM, including collagen type I and \(\alpha\)-SMA. The initiation stage is followed by the perpetuation stage, in which increased \(\alpha\)-SMA and collagen type I are amplified by enhanced growth factor expression, including TGF-\(\beta\)1.\(^{39}\) Alpha-SMA is a definite marker of transdifferentiation of stellate cells.\(^{40}\)

Our experiment shows that CC reduces \(\alpha\)-SMA and PDGFR-\(\beta\) expression in HSC-T6 cells. The results strongly indicate that CC alleviates hepatic fibrosis by blocking PDGFR-\(\beta\) and TGF-\(\beta\)1 signals. It has also been found that CC regulates \(\alpha\)-SMA expression by various means, including inhibition of HSC activation. In other words, CC might regulate liver fibrosis by inhibiting HSC activation.

Chronic liver disease develops via excessive accumulation of extracellular matrix proteins, including collagen.\(^{41}\) Generally, a normal liver can be sustained by ECM homeostasis. During liver fibrosis and cirrhosis, excessive synthesis of ECM proteins deteriorates the hepatic architecture.\(^{42,43}\) As a result, homeostasis of ECM is broken and TIMP-1 expression is increased.\(^{44}\) Homeostasis of ECM is maintained by the action of a specific system of proteolytic enzymes known as matrix metalloproteinase (MMP) and TIMP.\(^{45}\) TIMP-1 plays an important role in the progression of liver fibrosis.\(^{40,45}\) TIMP-1 expression is induced by a variety of stimuli, including PMA, TGF-\(\beta\), serum, retinoids, and interleukin-6/ Oncostatin M.\(^{46}\) On TIMP-1 Western blotting assay, CCE reduced the protein level of PMA-induced TIMP-1 expression in the HSC-T6 cells. This means that CCE can help in the homeostasis of ECM, as well as inhibit HSC activation, preventing liver fibrogenesis.

High-dose DMN treatment caused acute liver injury, leading to shrunken livers, accumulation of hydroxyproline, and biological changes.\(^{47}\) Recently, high-dose DMN-induced liver injury in rats is used as a research model for acute liver injury and the early stage of liver fibrosis.\(^{47}\) Hence this study investigated the effects of CC on acute liver injury using the DMN-induced liver injury model. Clinically, if hepatic disease is in doubt, LFTs (liver function tests) can be done. Because GOT and GPT are enzymatic indicators of tissue damage by toxicants or disease conditions, abnormal levels of GOT and GPT in the plasma are important to clinical pathology and toxicology.\(^{48}\) In all the control groups at 4 after high-dose DMN treatment, the levels of GOT, GPT, ALP, and other protein markers in the serum were significantly elevated or lowered and the liver was shrunken, while hydroxyproline accumulated in the liver. These results correspond to those of previous studies.\(^{47,49,50}\) In the acute liver injury model, CC administration showed restorative effects on serum T-protein, albumin, T-Bili, D-Bili, GOT, GPT, and ALP. In our study, we obtained a significant result that revealed that CC restored LFT in acute liver injury in vivo. This implies that CC can improve the function and condition of a liver with acute toxic injury. Cirrhotic livers revealed strong \(\alpha\)-SMA positivity in fibrous septae as well as in the perisinusoidal space of intact hepatocytes at the leading edge of fibrosis.\(^{51}\) Hydroxyprolin and \(\alpha\)-SMA showed no difference, as compared to the DMN-treated group in vivo. It is assumed that since treatment of rats with high-dose DMN for 4 was used in the acute hepatic injury model, fibrotic change might not occur in the liver and CC might have no room to affect fibrosis. We assume that our experimental animal model was an acute hepatic injury model due to
the use of high-dose DMN for 4, so the fibrotic change in the liver was not sufficient and CC did not affect α-SMA and hydroxyproline significantly. Therefore, we suggest that the chronic effects of CC against liver injury in vivo ought to be examined further.

Acknowledgments

This research was supported by the Program of Kyung Hee University for the Young Researcher in Medical Science (KHU-20071483).

References