Inhibitory Effect of 1-*O*-Hexyl-2,3,5-Trimethylhydroquinone on Dimethylnitrosamine-induced Liver Fibrosis in Male SD Rats

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Hepatic fibrosis represents the main complication of most chronic liver disorders and, regardless of its etiology, is characterized by excessive deposition of extracellular matrix components. In this study, we examined that 1-*O*-Hexyl-2,3,5-Trimethylhydroquinone (HTHQ), a potent anti-oxidative agent, could prevent experimental hepatic fibrosis induced by dimethylnitrosamine (DMN) in male SD rats. Except for vehicle control group, other groups were induced hepatic fibrosis by intraperitoneal injection with DMN (10 mg/ml/kg) on 3 consecutive days weekly for 4 weeks. During the same 4 weeks, control and DMN groups were given vehicle and HTHQ 50, 100 and 200 groups were orally administered HTHQ (50, 100, 200 mg/kg respectively). In HTHQ 100 and 200 groups, relative liver weight and serum chemistry level improved significantly. HTHQ reduced hydroxyproline ($p < 0.05$) and malondialdehyde ($p < 0.05$) level in the liver. Histopathological examination of H&E, Masson's trichrome stain showed the reduced fibrotic septa in HTHQ 100 and 200 groups. HTHQ administration showed reduced mRNA level of PDGF (Platelet-derived growth factor), α-SMA (α-smooth muscle actin) and TGF-β (transforming growth factor-β) than DMN-induced hepetic fibrosis animals in the liver tissue. In this study, we showed that HTHQ improves against DMN-induced liver fibrosis in male SD rats.

Key words: HTHQ, DMN, Hepatic fibrosis

INTRODUCTION

The liver plays a major role in metabolism and has a number of functions in the body, including glycogen storage, decomposition of red blood cells, plasma protein synthesis, and detoxification. It also performs and regulates a wide variety of high-volume biochemical reactions requiring very specialized tissues (Maton, 1993). Viral infection, alcoholic or drug toxicity, or any other factors that cause damage to hepatocytes exterts an inflammatory reaction in the liver. The damaged hepatocytes, their membrane components, metabolites of toxic agents, and infiltrating inflammatory cells are the activators of Kupffer cells. The activated Kupffer cells release a number of soluble agents, including cytokines, reactive oxygen species and other factors (Strain, 1998). These factors act on the hepatic stellate cells (HSCs) localized in the para-sinusoidal space and storing most of the vitamin A in the body. HSCs have two known functions: primary storage of the body's retinoids and the production of extracellular matrix components (ECM) when the liver is injured. Following liver injuries by any etiology, HSCs undergo a response known as "activation", which is the transition of quiescent cells into proliferative, fibrogenic, and contractile myofibroblasts (Shiba *et al.*, 1998; Shimizu *et al.*, 1999). HSCs activation is a remarkably pleiotropic yet tightly programmed response occurring in a reproducible sequence (Friedman, 2000). This sequence consists of 'initiation' and 'perpetuation'. Initiation encompasses rapid changes in gene expression and phenotypes that render the cells responsive to cytokines and other local stimuli. Perpetuation involves key phenotypic responses mediated by increased cytokine effects and remodeling of ECM (Friedman, 2000). Discrete phenotype responses of HSCs perpetuation include: 1) proliferation; 2) contractility; 3) fibrogenesis; 4) matrix degradation; 5) chemotaxis; 6) retinoid loss; and 7) cytokine release and white blood cell chemoattraction. Among these, 'proliferation' is to increase the number of HSCs in injured liver, which arise in part from local proliferation in response to polypeptide growth factors. Several

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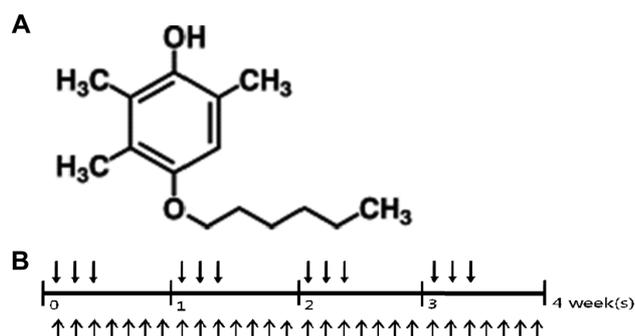
Fig. 1. Materials and methods. A. chemical structure of HTHQ, B. Experimental design. DMN (↓) was injected intra-peritoneally on three consecutive days weekly for 4 weeks to all rats, except for vehicle control group. During the same 4 weeks, the rats were orally administered with either HTHQ or vehicle (↑) for vehicle control group and DMN + vehicle group.

peptides and signal transduction molecules including platelet derived growth factor (PDGF) (Marra *et al.*, 1997), nuclear factor-κB (NF-κB) (Elsharkawy *et al.*, 1999), transforming growth factor-β (TGF-β), tumor necrosis factor-α (TNF-α) (Saile *et al.*, 1999) and phosphoinositol 3-kinase (Lang *et al.*, 2000) have been suggested to contribute to the regulation of HSCs proliferation (Pinzani *et al.*, 1998). And HSCs express α-smooth muscle actin (α-SMA) and desmin, a maker usually found in muscle cells (McGavin, 2007).

1-*O*-Hexyl-2,3,5-trimethylhydroquinone (HTHQ) (Fig. 1A), a lipophilic phenolic antioxidant, possesses strong antioxidant activity, even at low dose levels (Nihro *et al.*, 1994). This compound dose-dependently inhibits 2-amino-6-methyldipyrido[1,2-a:3',2'-d]imidazole (Glu-P-1) or 2-amino-1-methyl-6-phenylimidazo[4,5-b]-pyridine (PhIP)-induced mutagenesis in Salmonella TA98 in the presence of S-9 mixture (Hirose *et al.*, 1995c). Simultaneous treatment with HTHQ potently inhibited PhIP-induced mammary carcinogenesis in female rats without prior initiation treatment (Hirose *et al.*, 1995a), and Glu-P-1 or 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx)-induced hepatocarcinogenesis in male rats after initiation with N-diethylnitrosamine (DEN) (Hirose *et al.*, 1995b). The inhibitory effects of HTHQ on MeIQx-induced hepatocarcinogenesis were greater than those observed for naturally occurring and synthetic antioxidants (Hirose *et al.*, 1998). Therefore whether it might also influence hepatic fibrosis induced by DMN was one question targeted in the present study.

Dimethylnitrosamine (DMN) is a potent hepatotoxin, carcinogen and mutagen (Haggerty and Holsapple, 1990). At doses as small as 20 mg/kg, DMN can cause massive liver necrosis and death in many species (Hashimoto *et al.*, 1989). Exposure to repeated lower doses of DMN causes subacute and chronic liver injury with varying degrees of necrosis, fibrosis, and nodular regeneration (Magee and Barnes, 1967). DMN-induced fibrosis model is known to reproduce most of the features observed during human liver fibrosis (Bertelli *et al.*, 1996). This model has benefits, such as progressive and remarkable pathological alteration, a high reproduction rate of fibrosis and a low mortality rate in experimental animals (Jezequel *et al.*, 1989). An experimental liver fibrosis model induced by DMN in rats has been used in this study. In view of the protective effects of HTHQ against a variety of toxicants and carcinogens, this study was designed to determine whether HTHQ has an antifibrogenic effect on DMN-induced hepatic fibrosis in rats.

MATERIALS AND METHODS

Materials.   DMN and other reagents including hydroxyproline, sodium hydroxide, *p*-dimethylaminobenzaldehyde, chloramine-T, trimethoxypropane (TMP) were purchased from Sigma (St. Louis, MO, USA). HTHQ, Tween-80 and eco-rubber were supplied by the Nihon Hypox Co. (Yamanashi, Japan). Sodium dodecyl sulfate (SDS) was purchased from BioShop (Burlingston, Canada). Thiobarbituric acid (TBA) was purchased from Tokyo Chemical Industry Co. Ltd (Tokyo, Japan).

Animals.   Specific pathogen-free 6-week-old male Sprague Dawley (SD) rats were purchased from a commercial animal breeder (Orient Bio Inc., Gyeongido, Korea). Forty eight rats were acclimated for 1 week and housed in and environmentally controlled room at $22 \pm 2^{\circ}C$, and a 12 h light/dark cycle, and were provided commercial pellets (Purina Co., Korea) and tap water *ad libitum*. Our animal experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animal published by the U.S. National Institutes of Health.

Induction of liver fibrosis and administration of HTHQ.   Forty SD rats were randomly divided into five groups of eight animals. DMN (10 mg/ml/kg) was intra-peritoneally injected to all rats on three consecutive days weekly for 4 weeks, except for vehicle control group. During the same 4 weeks, the rats were orally administered with either HTHQ (50, 100 or 200 mg/kg) or vehicle (tween 80 and eco-rubber) for vehicle control group and DMN + vehicle group. Changes in body weight were recorded weekly throughout the experimental period. The animals were sacrificed on the 28th day and liver was isolated and weighed to calculate its relative weight to the final body weight. Blood samples for biochemical analysis were obtained from the abdominal aorta. Liver tissue was separately stored for histopathological examination, RNA expression analysis and measurement of hydroxyproline and malondialdehyde (MDA).

Serum biochemical analysis.   Total blood was collected via the abdominal aorta after ether anesthesia on the final day of the experiment. Following blood clotting, the serum

Table 1. The sequence of primers was used in this study

Gene name	Forward primer	Reverse primer
β-actin (539 bp)	5'-GTG GGG CGC CCC AGG CAC CA-3'	5'-CTC CTT AAT GTC ACG CAC GAT TTC-3'
TGF-β (314 bp)	5'-TGA GTG GCT GTC TTT TGA CG-3'	5'-TTC TCT GTG GAG CTG AAG CA-3'
PDGF-β (229 bp)	5'-CTG CCT CTC TGC TGC TAC CT-3'	5'-GAT GAG CTT TCC GAC TCG AC-3'
α-SMA (247 bp)	5'-CAT CAG GAA CCT CGA GAA GC-3'	5'-TCG GAT ACT TCA GGG TCA GG-3'

levels of asparate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyltranspeptidase (γ-GT), alkaline phosphatase (ALP), total bilirubin, total cholesterol, triglyceride, total protein, albumin and albumin/globulin ratio (A/G ratio) were measured by Hitach Clinical Analyzer 7080 (Hitachi Medical, Tokyo, Japan).

Hepatic hydroxyproline content.   The hepatic hydroxyproline content was determined using a modification of the method reported by Reddy and Enwemeka (1996). Briefly, 0.2 g liver tissue stored at −70°C were homogenized in 2 m*l* distilled water. Aliquots of standard hydroxyproline (2~20 μg) and test samples were mixed gently with sodium hydroxide (2 N final concentrations) in a total volume of 50 μ*l*. The samples were hydrolyzed by autoclaving at 120°C for 20 minute. 450 μ*l* of chloramine-T was added to the hydrolyzate, mixed gently, and the oxidation was allowed to proceed for 25 min at room temperature. 500 μ*l* of Ehrlich's aldehyde reagent was added to each sample, mixed gently, and the chromophore was developed by incubating the samples at 65°C for 20 min. Absorbance of each sample was read at 550 nm using a spectrophotometer and compared to the value from prepared hydroxyproline standard curve. Protein concentrations were determined according to the method of Lowry *et al*. (1951), using bovine serum albumin (BSA) as a standard.

Hepatic malondialdehyde (MDA) determination.   Lipid peroxidation levels in the liver tissue were measured using the method of thiobarbituric acid reactive substances (TBARS). The concentration of TBARS was expressed as nM MDA/mg protein using TMP as a standard. Briefly, 0.2 g liver tissue was homogenized in 1.8 m*l* ice-cold 10 mM phosphate buffer and 1 m*l* homogenate was mixed with 1 m*l* 8.1% SDS solution and 2 m*l* 20% acetic acid solution. After adding 1 m*l* 0.75% TBA solution to this mixture, it was heated for 30 minute in 95°C oven and then cooled at room temperature and centrifuged at 3500 rpm. for 15 min. The absorbance of the upper layer was measured at 532 nm with a spectrophotometer and compared to the value from prepared TMP standard curve. Protein concentrations were measured according to the method Lowry *et al*. (1951), using BSA as a standard.

Reverse transcriptase-PCR.   Total RNA was extracted from liver tissue samples using RNAlater (Ambion, Austin, TX, USA) and a RNA easy column (Qiagen, Valencia, CA,

USA), following homogenization in TRI Reagent (Molecular Research Center). Same amounts from three samples was mixed and used to cDNA synthesis. cDNA was synthesized using 10 pmol oligo dT (Bioneer, Daejeon, Korea). After incubation at 70°C for 10 min, 2.5 mmol/*l* dNTP mixture (Bioneer) and 200 U/μ*l* reverse transcriptase (Promega, Madison, WI, USA) were added in a final volume of 20 μ*l*. After cDNA synthesis, PCR was performed using Go Taq polymerase (Promega) with the primers. The sequence of primers was described in Table 1. The reactions were performed with 0.2 μ*l* Go Taq polymerase (5 U/μ*l* Promega), 1 μ*l* 10 pmol primer pairs, 3 μ*l* 10 pmol dNTP, 6 μ*l* 5 × reaction buffer, 19.8 μ*l* distilled water, and 3 μ*l* cDNA diluted three times with distilled water. PCR was performed for 27 to 35 cycles under the following conditions: initial denaturation at 95°C for 5 min, denaturation for each cycle at 95°C for 1 min, annealing at 58°C for 40 sec, and amplication at 72°C for 40 sec. After PCR, electrophoresis was performed on agarose gel.

Histopathological examination.   The liver was removed, and fixed in Bouin's solution. For histological examination, paraplast-embeded liver tissues were sectioned (4 μm thick), were stained with Hematoxylin-Eosin (H&E) and Masson's trichrome. The tissue slides were photographed at 20 and 100 magnifications.

Statistical analysis.   Data are expressed as mean ± SD. Statistical significance between the vehicle control and DMN + vehicle group, and DMN + vehicle group and DMN + HTHQ treated groups were determined by one-way analysis of variance (ANOVA) followed by the Tukey and Duncan test using the SPSS 12.0 statistics computer program. A difference in the mean values of $p < 0.05$ was considered to be statistically significant.

RESULTS

The changes in body weight and liver weight.   DMN treatment had significantly decreased body weight ($p < 0.05$). While there were no significant differences between DMN group and HTHQ treated groups in body weight, 4 week-body weight of HTHQ 100 and 200 groups showed a tendency to increase (data not shown). In addition, DMN treatment had significantly decreased the relative liver weight compare to vehicle control group as shown Fig. 2A. Oral
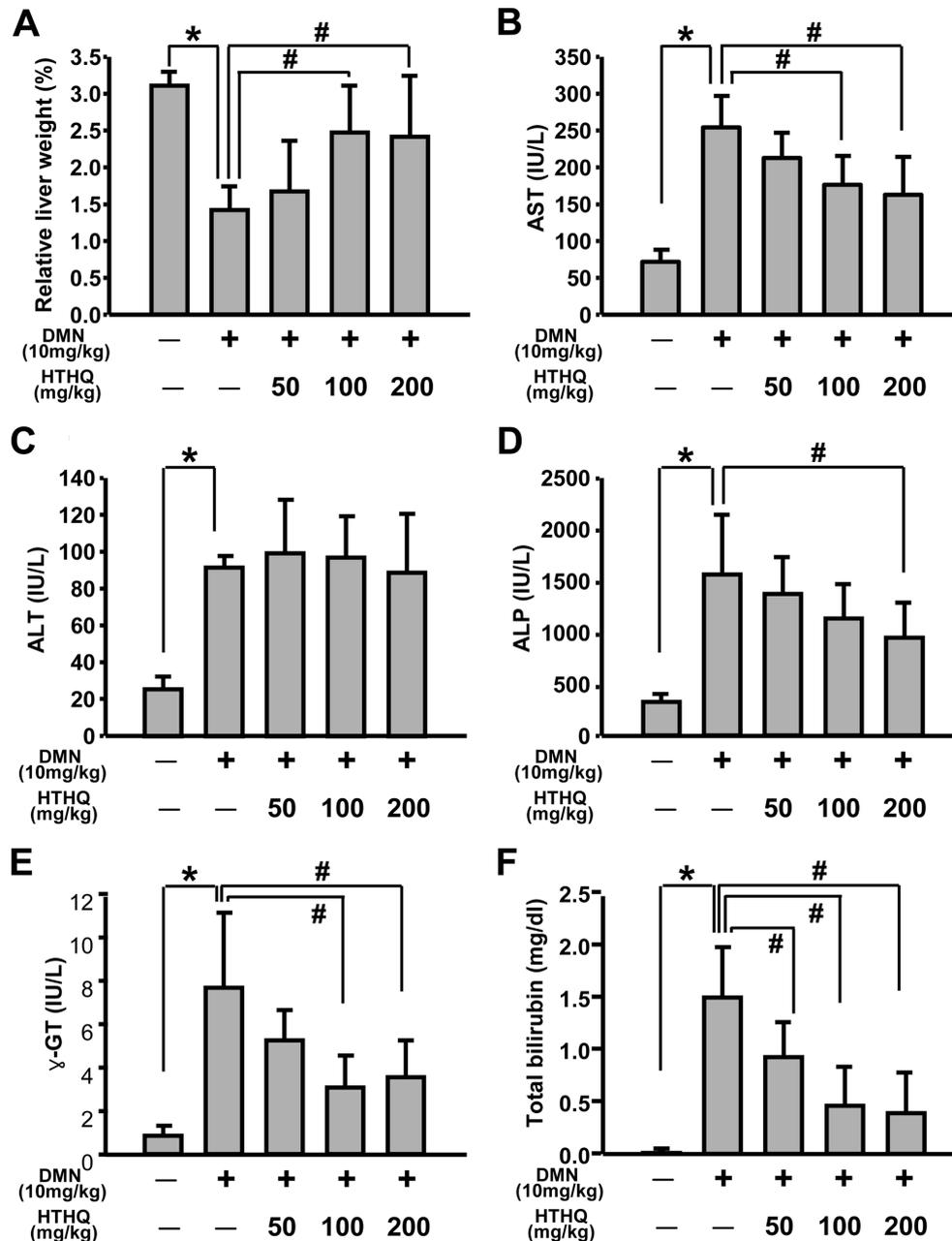
Fig. 2. Systemic changes about HTHQ effects on DMN-induced hepatic damage. A. relative liver weights, B. serum AST levels, C. serum ALT levels, D. serum ALP levels, E. serum γ-GT levels, F. total bilirubin levels in the serum. Data were plotted in the graph as mean ± SD (n = 8). *; Significant difference form vehicle control group (p < 0.05). #; Significant difference form DMN + vehicle group (p < 0.05).

HTHQ 100 or 200 mg/kg treatment increased this DMN-induced relative liver weight loss significantly (p < 0.05).

Serum biochemistry. The effects of HTHQ on serum parameters in the liver fibrosis model are shown in Fig. 2. B~F. Serum AST was elevated by DMN treatment (253 IU/*l* versus 71 IU/*l*) (p < 0.05) and impaired by HTHQ 100 and 200 to 175 IU/*l* and 162 IU/*l* respectively (p < 0.05). Serum

ALT significantly increased in DMN group (90 IU/*l* versus 25 IU/*l*) (p < 0.05) but HTHQ did not affect to the level of ALT increased by DMN. Serum ALP was significantly raised by DMN treatment (1545 IU/*l* versus 323 IU/*l*) (p < 0.05) and the levels in HTHQ 200 group (939 IU/*l*) was significantly lower than that in the DMN group (p < 0.05). Serum γ-GT was significantly elevated by DMN treatment (7.6 IU/*l* versus 0.8 IU/*l*) (p < 0.05) and the levels in HTHQ 100 and

200 groups were significantly lower than that in the DMN group (3.0 IU/l and 3.5 IU/l, respectively) (p < 0.05). Total bilirubin significantly increased in DMN group (1.49 mg/dl versus 0.01 mg/dl) (p < 0.05) and the levels in HTHQ 50, 100 and 200 groups decreased dose-dependently and signif-

icantly than that in the DMN group (0.92, 0.46 and 0.39 mg/dl, respectively) (p < 0.05).

Hydroxyproline and MDA contents in liver tissues.   For measurement of collagen, hydroxyproline contents of each
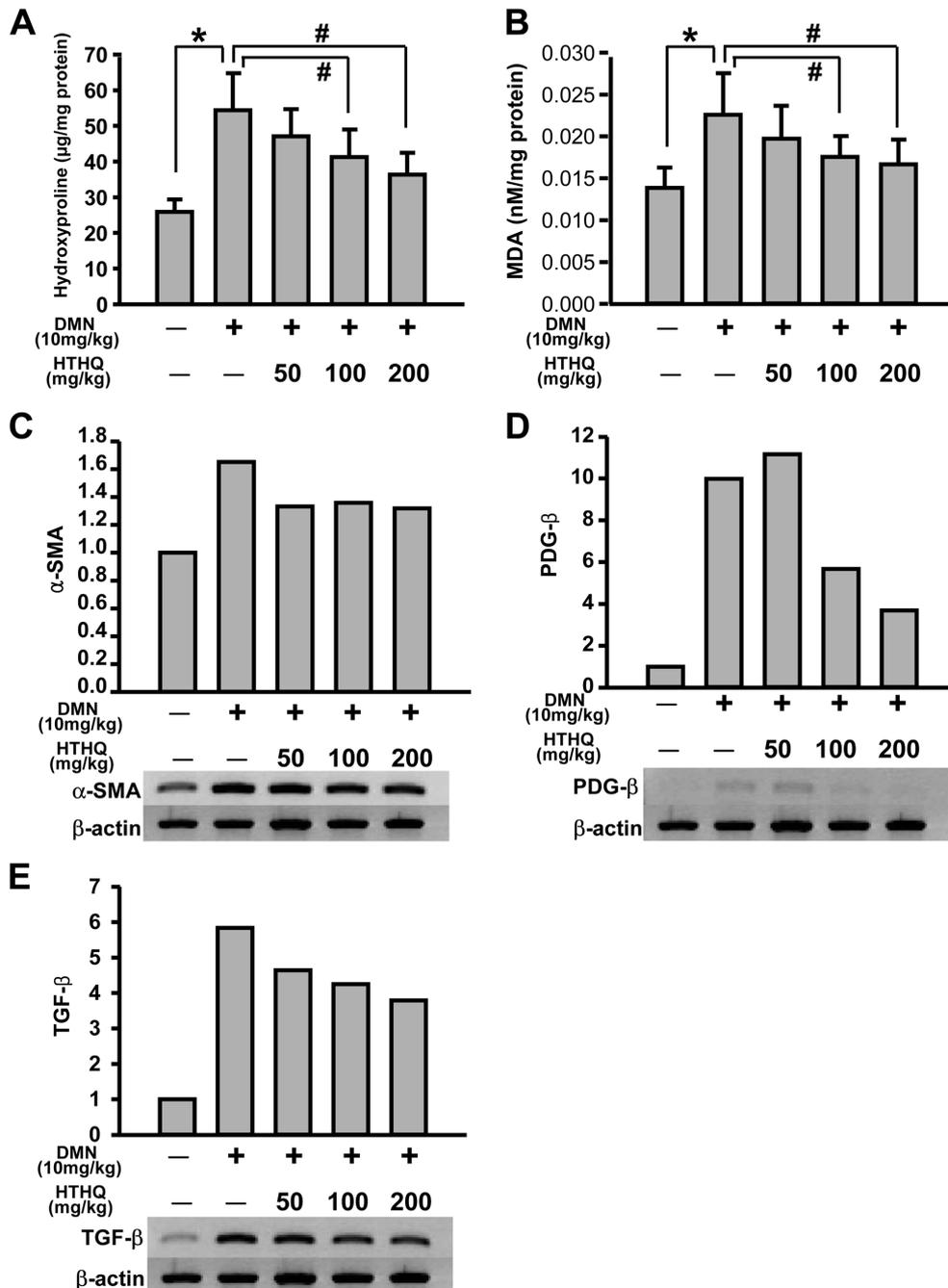

Fig. 3. Molecular changes in liver tissues about HTHQ effects on DMN-induced hepatic damage. A. Hydroxyproline content levels, B. MDA content levels, C. α-SMA gene expression, D. PDGF-β gene expression, F. TGF-β gene expression. The data of C, D, E and F are represented as inverted images and relative gene expression which is the percentage of gene expression each group to that in control group after normalization to β-actin gene expression. Data were plotted in the graph as mean ± SD (n = 8). *; Significant difference form vehicle control group (p < 0.05). #; Significant difference form DMN + vehicle group (p < 0.05).

liver were analyzed as showed Fig. 3A. The significantly increased deposition of hepatic collagen in the DMN group (54.32 ± 9.94 μg/mg protein) compared to control group (25.89 ± 3.22 μg/mg protein) was shown ($p < 0.05$). Hepatic collagen deposition in HTHQ treated groups dose-dependently decreased. Also, in DMN + HTHQ 100 and 200

groups (41.21 ± 7.50 and 36.31 ± 6.12 μg/mg protein) there are significant decreases ($p < 0.05$).

The lipid peroxidation in the liver was measured by MDA determination in liver homogenates. As shown in Fig 2B. the MDA contents in DMN + vehicle group (0.0226 ± 0.0049 nM/mg protein) was significantly higher than that of
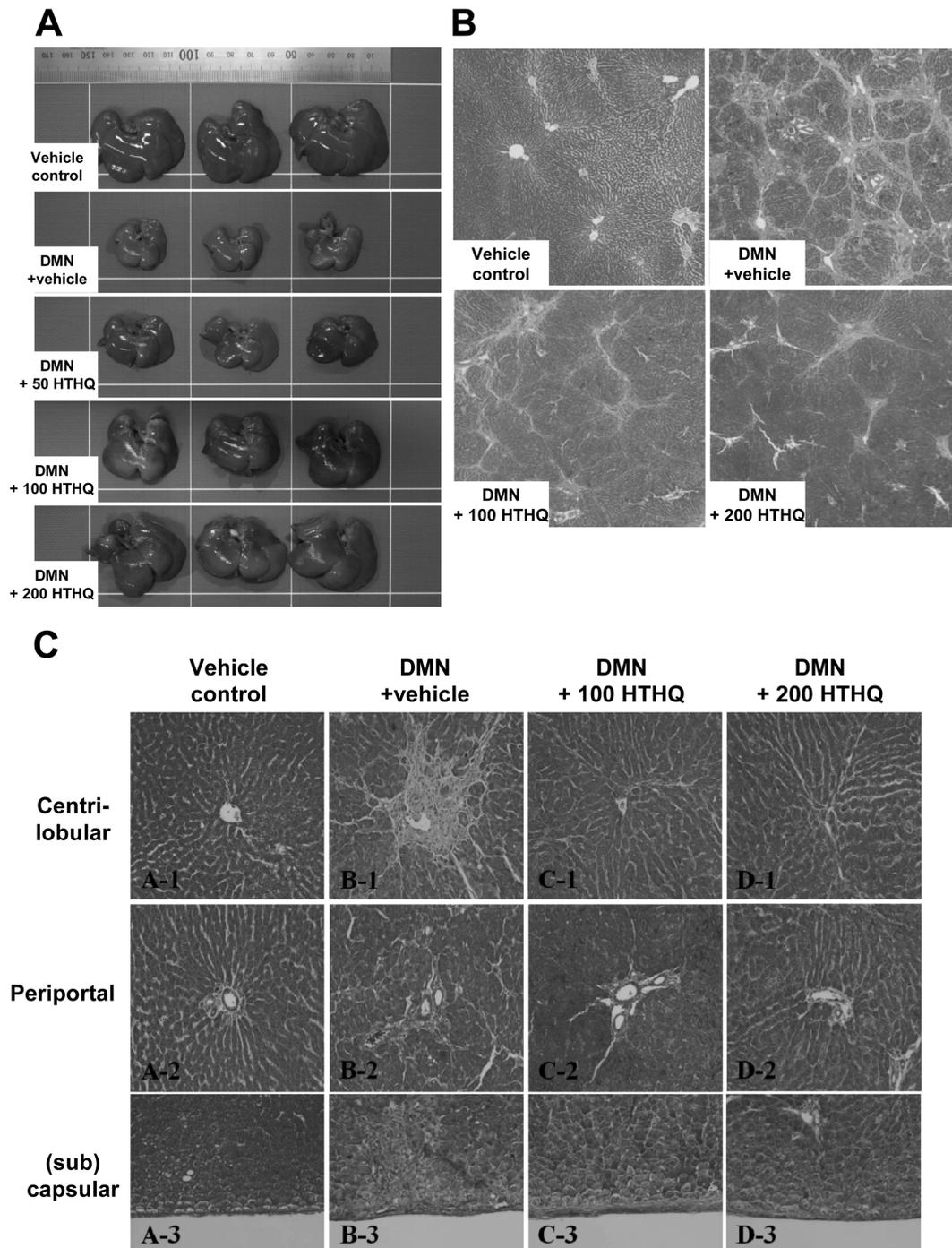
Fig. 4. Gross and histopathological features of the liver. A. gross features of the liver, B and C. histopathological feature on Masson's trichrome staining liver slides.

the control group (0.0138 ± 0.0024 nM/mg protein) ($p < 0.05$). But treatment of HTHQ 100 and 200 mg/kg decreased to 0.0175 ± 0.0022 and 0.0166 ± 0.0030 nM/mg protein, respectively.

Gene expression analysis.   To investigate other molecular mechanism of HTHQ, it analyzed the expression of several fibrosis-associated genes, α-SMA, PDGF-β and TGF-β in liver tissues (Fig. 3C~E). Data is represented as inverted images and relative gene expression which is the percentage of gene expression each group to that in vehicle control group after normalization to β-actin gene expression. The gene expression of α-SMA, PDGF-β and TGF-β up-regulated by 1.65, 9.99 and 5.84, respectively, in DMN group. HTHQ treatment (50, 100, 200 mg/kg) down-regulated α-SMA gene expression to 1.33, 1.36 and 1.32, respectively. PDGF-β gene expression also decreased to 5.67 and 3.68 in HTHQ 100 and 200, respectively. TGF-β gene expression was dose-dependently down-regulated to 4.64, 4.25 and 3.80, respectively, in HTHQ treated groups.

Gross and histopathological findings.   Intraperitoneal administrations of DMN for 4 weeks resulted in considerably down-sized liver (Fig. 4A-DMN + vehicle) but these changes were inhibited by the HTHQ treatment (Fig. 4A-DMN + 100, 200 HTHQ).

To investigate fibrotic changes in the liver, Masson's trichrome stain was used. Microscopic analysis revealed cirrhosis-like structural patterns in the DMN + vehicle group: that is, fibrous connective tissue components in pseudo-lobule formations and formation of fibrotic septa and thickened reticulin fibers (Fig. 4B DMN + vehicle). Also, bridging fibrosis (central-cental, central-portal and portal-portal) (Fig. 4B DMN + vehicle) was observed. Thickening of the Glisson's capsule and collagen deposition of subcapsular area back to the capsule were shown (Fig. 4C-DMN-vehicle, subcapsular). Fibrosis in both the centri-lobular (Fig. 4C-centri-loblar) and periportal (Fig. 4C-DMN-vehicle, periportal) area was shown in DMN group. But HTHQ 100 and 200 mg/kg treatment showed diminished fibrosis in both the centri-lobular (Fig. 4C-centri-loblar, DMN-100, 200 HTHQ), periportal (Fig. 4C-periportal, DMN-100, 200 HTHQ), Glisson's capsule and subcapsular (Fig. 4C-subcapsular, DMN-100, 200 HTHQ) area.

DISCUSSION

Hepatic fibrosis represents the main complication of most chronic liver disorders and, regardless of its etiology, is characterized by excessive deposition of extracellular matrix components (Friedman *et al.*, 1985). The main collagen-producing cells of the liver are HSCs, which, when activated, turn into myofibroblast-like cells with a markedly increased capacity for collagen synthesis (Friedman, 1990;

Gressner and Bachem, 1990).

HTHQ, a lipophilic phenolic antioxidant has very strong antioxidant activity (Nihro *et al.*, 1994). It has reported to have several effects, such as mutagenesis inhibition effect (Hirose *et al.*, 1995c), mammary carcinogenesis suppression effect (Hirose *et al.*, 1995a) and hepatocarcinogenesis inhibition effect (Hirose *et al.*, 1995b, 1998). Whether it might also influence hepatic fibrosis induced by DMN was one question targeted in the current study.

DMN is a potent hepatotoxin, carcinogen and mutagen (Haggerty and Holsapple, 1990). DMN-induced fibrosis model is known to reproduce most of the features observed during human liver fibrosis (Bertelli *et al.*, 1996). This model has benefits, such as progressive and remarkable pathological alteration, a high reproduction rate of fibrosis and a low mortality rate in experimental animals (Jezequel *et al.*, 1989). Although the intimate mechanism of DMN-induced liver fibrosis is not completely clarified, it is known that DMN must be metabolized by the cytochrome P-450 system before it becomes active, both chemically and biologically (Farber, 1996). Also, it has been shown that DMN-reactive metabolites may contribute to the toxic effects in P-450 2E1-expressing cells (Li *et al.*, 1990). In view of the protective effects of HTHQ against a variety of toxicants and carcinogens, this study is designed to determine whether HTHQ has an anti-fibrogenic effect on DMN-induced hepatic fibrosis in rats.

Liver function tests are widely used examinations to measure the condition of the liver. Aminotransferases including ALT and AST are enzymes located in liver cells that leak out into the general circulation when liver cells are injured (Ahmed and Keeffe, 2004). Serum AST and ALP were significantly elevated by DMN treatment (Britton and Bacon, 1994). AST level decreased by HTHQ 100, 200 mg/kg treatment but ALT had no significant differences between DMN and HTHQ treated group. ALP and γ-GT are enzymes which are used to judge the biliary tract damage. Serum ALP and γ-GT were significantly raised by DMN treatment ($p < 0.05$) and the levels decreased dose-dependently by HTHQ treatment. Serum bilirubin is the most direct indexes to examine metabolic state of the liver (Mancuso *et al.*, 2006). Increased bilirubin level induced by DMN treatment dose-dependently and significantly reduced by HTHQ treatment ($p < 0.05$). In this research it could be found that HTHQ could well reduce jaundice. It could be inferred that HTHQ had a protective effect on biliary tract damage.

For measurement of collagen, hydroxyproline contents of each liver were analyzed. The hepatic collagen deposition in DMN treated group significantly increased ($p < 0.05$) and HTHQ reduced these dose-dependently changes. This suggests that HTHQ has an anti-fibrogenic effects in DMN-induced hepatic fibrosis.

MDA is one of the most frequently used indicators of lipid peroxidation (Baskol *et al.*, 2007). The MDA contents

in DMN group was higher than that of the control vehicle group (162% of control) and the HTHQ 100 and 200 groups, it provided 36% and 42% of protection, respectively. This data shows that the DMN-treated rats exhibited increased levels of hepatic MDA and that HTHQ reduced this increase. This result suggests that the mechanism for the hepato-protective effect of HTHQ may be related to the reduction of lipid peroxidation.

To investigate other possible molecular mechanisms of HTHQ's effects, we analyzed the expression of several fibrosis-associated genes in liver tissue. HTHQ treatment revealed the reduced mRNA level of α-SMA, PDGF-β and TGF-β. In the progression stage of liver, Kuffer cells are activated and secrete pro-inflammatory cytokines, which stimulate HSCs (Marra *et al.*, 1997; Pinzani *et al.*, 1998; Saile *et al.*, 1999). Kuffer cells are also activated by toxins such as DMN and CCl₄ (Edwards *et al.*, 1991; Luckey and Petersen, 2001). Activated macrophage-derived pro-inflammatory cytokines such as PDGF-β and TGF-β generally increase during the progression of fibrosis (Marra *et al.*, 1997; Saile *et al.*, 1999) These cytokines, which primarily originate from activated Kuffer cells and HSCs, cause the transformation of HSCs into myofibroblast, which secrete extracellular matrix and release α-SMA. In this study, the gene expression of α-SMA, PDGF-β and TGF-β up-regulated by DMN treatment but which was down-regulated by HTHQ treatment.

In histological examination of tissue slide stained by H&E and Masson's trichrome, DMN treatment increased formation of fibrotic septa, bridging fibrosis and liver collapse such as capsular and sub-capsular fibrosis (Fig. 4B and 4C). Bridging necrosis is an indication of severe liver damage, and is associated with poor prognosis (Cooksley *et al.*, 1986; Trump and Berezesky, 1992). In contrast, the livers of rats that received HTHQ showed mild bridging fibrosis, diminished fibrosis in both the peripotal and centri-lobular liver and reduced deformation of the liver acinus. In conclusion, our results suggest that HTHQ have inhibitory effect against liver fibrosis.

ACKNOWLEDGMENT

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