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A novel synthetic oleanolic acid derivative (CPU-II_2) attenuates liver fibrosis in mice through regulating the function of hepatic stellate cells

Li-Mei Wu[1], Xing-Xin Wu[1], Yang Sun[1], Xiang-Wen Kong[2], Yi-Hua Zhang[2,*] & Qiang Xu[1,*]

[1]*State Key Laboratory of Pharmaceutical Biotechnology, School of Life Sciences, Nanjing University, 22 Han Kou Road, Nanjing, 210093, China;* [2]*Center of Drug Discovery, China Pharmaceutical University, 24 Tongjiaxiang, Nanjing, 210009, China*

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Abstract

Regulation on the function of the hepatic stellate cells (HSCs) is one of the proposed therapeutic approaches to liver fibrosis. In the present study, we examined the in vitro and in vivo effects of CPU-II_2, a novel synthetic oleanolic acid (OLA) derivative with nitrate, on hepatic fibrosis. This compound alleviated CCl_4-induced hepatic fibrosis in mice with a decrease in hepatic hydroxyproline (Hyp) content and histological changes. CPU-II_2 also attenuated the mRNA expression of α-smooth muscle actin (α-SMA) and tissue inhibitor of metalloproteinase type 1 (TIMP-1) induced by CCl_4 in mice and reduced both mRNA and protein levels of α-SMA in HSC-T6 cells. Interestingly, CPU-II_2 did not affect the survival of HSC-T6 cells but decreased the expression of procollagen-α1 (I) in HSC-T6 cells through down-regulating the phosphorylation of p38 MAPK. Conclusion: CPU-II_2 attenuates the development of liver fibrosis rather by regulating the function of HSCs through p38 MAPK pathway than by damaging the stellate cells.

Introduction

Liver fibrosis is a wound-healing process that is elicited by various toxic stimuli, and it is characterized by an excessive deposition of extracellular matrix (ECM) proteins that type I collagen predominates [1]. As the main source of accumulated ECM, activated hepatic stellate cells (HSCs), which are proliferative and fibrogenic, have been evidenced to play a key role in the development of liver fibrosis [2, 3]. Such activated HSCs show a character with high expression of α-smooth muscle actin (α-SMA) [4, 5], a key marker of the HSCs activation. The degradation for the accumulated ECM from activated HSCs is essential for the alleviation of fibrosis, which is catalyzed by matrix metalloproteinases (MMPs). In contrast, tissue inhibitor of metalloproteinase type 1 (TIMP-1) can attenuate spontaneous resolution of liver fibrosis by reducing the MMP activity and suppressing the apoptosis of HSCs [6]. Thus, the induction of apoptosis for stellate cells has become one of the treatment strategies for liver fibrosis [2]. However, in physiological conditions, quiescent HSCs play important roles in the regulation of retinoid homeostasis and ECM remodeling by producing ECM components as well as metalloproteases and their inhibitors [7]. From this consideration, treatment by inducing apoptosis of HSC may lead to undesired effects.

Among various factors involved in the development of liver fibrosis [8], TGF-β is known as one of the most essential proteins. This cytokine could be secreted from activated HSCs, and its cell line,

*To whom correspondence should be addressed. Fax: +86-25-83597620; E-mail: molpharm@163.com (Q. Xu);
Fax: +86-25-86635503; E-mail: zyhtgd@hotmail.com (Y.H. Zhang)

HSC-T6 cells [4, 9]. TGF-β is able to induce the activation of p38 MAPK pathway, which is involved in the TGF-β-induced transcriptional activation by regulating the Smad-mediated pathway [10, 11]. MAPKs are important signal transduction enzymes, which may regulate many cellular physiological functions including gene expression, cell proliferation, and apoptosis. Four subgroups of the MAPKs family, ERK1/2, JNKs, and p38 proteins, have been identified [12–14]. A recent study has also shown that there is higher activation of basal p38 MAPK in activated HSCs than in quiescent HSCs [15], indicating the potential importance of p38 MAPK in the activation process of HSCs. In fact, many other studies have also proved the involvement of p38 MAPK pathway in the process of liver fibrosis.

Oleanolic acid (OLA), on the other hand, is a kind of clinical drug that commonly used for the treatment of acute and chronic hepatitis. This compound has been reported to have numerous pharmacological activities including attenuating hepatic inflammation and promoting regeneration of hepatic cells [16–18]. Since its low bioavailability and unsatisfying curative effects, many researchers have begun to synthesize various derivatives by adding new groups to expect more potent effects. Meanwhile ferulic acid is an antioxidant widely existent in plants. Based on these research backgrounds, a novel synthetic OLA derivative CPU-II_2 (Fig. 1) has been designed and synthesized by Prof. Yi-Hua Zhang, a co-author of this paper. In the synthesis, leading compound OLA was coupled with NO donors through ferulic acid as a linker to OLA-28-COOH [19]. The present study was designed to investigate the anti-fibrosis effect of CPU-II_2 and found that CPU-II_2 can rescue animals from hepatic fibrosis through inhibiting the deposition of extra cellular matrix (ECM) proteins and, especially through inhibiting the function of activated HSCs by down-regulating the p38 MAPK signaling pathway.

Materials and methods

Materials

CPU-II_2, 3-O-trifluoro-acetyl-Oleanolic-acid-2-methoxy-4-[2-(3-nitrooxy-propoxycarbonyl)-vinyl]-phenyl ester (Fig. 1) was synthesized by Prof. Yi-Hua Zhang at China Pharmaceutical University. Colchicine was purchased from Shanghai Kefeng Co. Ltd. (Shanghai, China). Kits for determining serum alanine transaminase (ALT), aspartate transaminase (AST), lactic dehydrogenase (LDH), and hydroxyproline (Hyp) were obtained from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). CytoTox 96 Nonradioactive Cytotoxicity assay was from Promega (Madison, WI). TGF-β1 was purchased from Peprotech (Rocky Hill, NJ). Antibodies against phospho-p38 and secondary antibodies of anti-mouse and rabbit IgG conjugated with horseradish peroxidase (HRP) were bought from Cell Signaling Technology (Cell Signaling Technology, MA). Antibodies to α-SMA and to α tubulin were from Santa Cruz (Santa Cruz Biotechnology, Inc, CA). Antibodies to Actin were from Boster (Wuhan, China).

Animal treatments

Male ICR mice (body weight 18–22 g) were supplied by the Experimental Animal Center of Nanjing Medical University (Nanjing, China). They were maintained with free access to pellet food and water in plastic cages at 21 ± 2 °C and kept in a 12-hour light-dark cycle. Animal welfare and experimental procedures were carried out strictly in accordance with the guide for the care

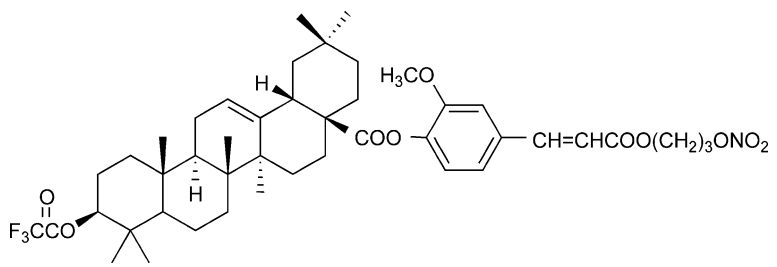

Figure 1. Chemical structure of CPU-II_2.

and use of laboratory animals (Ministry of Science and Technology of China, 2006) and the related ethical regulation of our university. All efforts were made to minimize animals' suffering and to reduce the number of animals used.

The mice were given 50 μmol/kg of OLA, 50 and 100 μmol/kg of CPU-II_2 subcutaneously or 6 μmol/kg of colchicine intragastrically daily for 8 weeks, respectively. Those in normal and control groups were given equal volume of vehicle solution. After one-week administration, all mice except for those in normal group were intraperitoneally injected with CCl_4 (0.8% CCl_4/olive oil 10 ml/kg body weight three times a week) for seven weeks. Then, the mice were sacrificed 24 h after the last injection by bleeding. The serum was collected and stored at −70 °C for the assays of ALT, AST and LDH. Liver and spleen were taken away, and weighted. Then livers were divided into three portions: (1) preserved in 10% formalin for histological examination, (2) frozen at −70 °C for Hyp assay and other biochemical assays, and (3) immediately used for total RNA isolation.

Morphometric collagen determination

The liver sections imbedded in paraffin were cut (5 μm) and stained with hematoxylin-eosin (H&E) and Masson's trichrome to determine the collagen distribution [20].

Hydroxyproline content

Hydroxyproline content in the liver was determined by the spectrophotometric method as Hyp assay kit's instruction manual [21]. The data was expressed as Hyp (mg)/wet liver weight (g).

Cell culture

HSC-T6 cells were purchased from Cancer Institute and Hospital, Chinese Academy of Medical Sciences. The cells were cultured in RPMI 1640 medium (Gibco, NY) supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin and 10% new bovine serum (Hangzhou Sijiqing Co., Ltd., Hangzhou, China), under a humidified 5% (v/v) CO_2 atmosphere at 37 °C. LX-2 cells were purchased from Xiangya Central Experiment Laboratory, Central South University, China. The cells were cultured in Dulbcco's Modifed Eagle Medium (DMEM) medium (Gibco, NY) supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin and 10% new bovine serum (Hangzhou Sijiqing Co., Ltd., Hangzhou, China), under a humidified 5% (v/v) CO_2 atmosphere at 37 °C.

Cell viability assay

To determine specific cell viability, we used the CytoTox 96 Nonradioactive Cytotoxicity assay (Promega) based on the calorimetric detection of the lactate dehydrogenase activity present in the cytoplasm of intact cells. HSC-T6 cells were cultured in 96-well plate at a density of 5×10^4 cells/ml in the media (0.2 ml) for 12, 24 and 48 h in the presence or absence of CPU-II_2 (10, 100, 1000 and 10000 nM) or colchicine (200 nM). Then, cell samples of interest were lysed by adding 15 μl of lysis solution (9% (v/v) Triton X-100 in water) per 100 μl of culture medium, followed by incubation at 37 °C for 50 min. Sample supernatants (50 μl) were then transferred to a fresh 96-well enzymatic assay plate. Reconstituted Substrate Mix (50 μl) was added to each supernatant sample, and the enzymatic reaction was allowed to proceed for 30 min at room temperature, protected from light. The enzymatic assay was then stopped by adding 50 μl/well of the stop solution. The plate was read at 490 nm using an ELISA plate reader.

Reverse transcriptase-polymerase chain reaction (RT-PCR) and real-time PCR

Total RNA was extracted from the liver tissues of the mice using Tripure reagent (Roche Diagnostics, Indianapolis, IN) as described by the manufacturer. Single-stranded cDNA was synthesized from 2 μg of total RNA by reverse transcription using 0.5 μg primer of oligo$(dT)_{18}$. Then the amplification was performed using the primers (Genebase, Shanghai, China) as follows: GAPDH (mouse) forward 5′-AACGACCCCTTCATTGAC-3′, reverse 5′-TCCACGACATACTCAGCAC-3′; α-SMA (mouse) forward 5′-CATCCACGAAACCACCTA-3′, reverse 5′-GGGCAGGAATGATTTGGA-3′; TIMP-1 (mouse) forward 5′-ACTCGGACCTGGTCATAAGGGC-3′, reverse 5′-TTCCGTGGCAGGCAAGCAAAGT-3′. The PCR cycle conditions were: 94 °C for 30 s, 58 °C for 45 s, and 72 °C for 30 s for 28 cycles, respectively. After amplification, PCR products were separated

by electrophoresis on 1.5% agarose gels and visualized by ethidium bromide dying. The relative expressions were quantified densitometrically using the LabWorks 4.0 software, and calculated according to the reference bands of GAPDH.

After incubation with CPU-II_2 (0.1, 1, 10 and 100 nM) for 24 h, HSC-T6 cells were harvested, and total RNA was extracted using Tripure reagent (Roche Diagnostics, Indianapolis, IN). Single-stranded cDNA was synthesized from 2 μg of total RNA by reverse transcription using 0.5 μg primer of oligo$(dT)_{18}$. Amplification was carried out in a total volume of 20 μl for 40 cycles of the same PCR condition mentioned above and product was detected using EvaGreen (Biotium, Inc., CA). Quantitative RT-PCR was done in the ABI 7000 Sequence Detection System (Applied Biosystems Inc.), by using the following primers: α-SMA (rat) forward 5′-GATCACCATCGGGAATGAACGC-3′, reverse 5′-CTTAGAAGCATTTGCGGTGGAC-3′; procollagen-α1 (I) (rat) forward 5′-CCTCAAGGGCTCCAACGAG-3′, reverse 5′-TCAATCACTGTCTTGCCCCA-3′; GAPDH (rat) forward 5′-GTGCTGAGTATGTCGTGGAG-3′, reverse 5′-GTCTTCTGAGTGGCAGTGAT-3′. Samples were run in triplicate, and their relative expression was determined by normalizing expression of each target to GAPDH, and then comparing this normalized value to the normalized expression in a reference sample to calculate a fold-change value.

Western blot analysis

HSC-T6 or LX-2 cells were seeded into 60 mm dishes at 1×10^6 cells/dish. In the next day, the HSC-T6 cells were treated with (0.01, 0.1, 1, 10 and 100 nM) CPU-II_2 for 48 h and LX-2 cells were treated with (1, 10 and 100 nM) CPU-II_2 in the presence of 2 ng/ml TGF-β1 for 48 h, then they were harvested and extracted in the lysis buffer consisting of 50 mM Tris–HCl, pH 8.0; 50 mM KCl, 5 mM DTT, 1 mM EDTA, 0.1% SDS, 0.5% Triton X-100 and protease inhibitor cocktail tablets (Roche, IN). The extracted proteins were separated by polyacrylamide/SDS gel and electrophoretically transferred onto polyvinylidene fluoride (PVDF) membranes (Roche, IN). The membranes were probed with antibodies overnight at 4 °C, and then incubated with a HRP coupled secondary antibody. Detection was performed using a LumiGLO chemiluminescent substract system (KPL, Guildford, UK). The relative expressions were quantified densitometrically using the LabWorks 4.0 software, and calculated according to the reference bands of α-tubulin or Actin.

Statistical analysis

Results were expressed as mean ± SEM. Statistically evaluated by Student's t test when only two value sets were compared, and one-way ANOVA followed by Dunnett's test when the data involved three or more groups. $P < 0.05$ was considered to be significant.

Results

Effects of CPU-II_2 on CCl_4-induced liver fibrosis in mice

As shown in Table 1, CCl_4-treated mice showed higher plasma ALT, AST and LDH levels as well

Table 1. Changes in various parameters in CCl_4-induced fibrotic mice with or without receiving CPU-II_2 (50 and 100 μmol/kg), Colchicine (6 μmol/kg) or vehicle treatment.

Group	Normal	Control	CPU-II_2 50 μmol/kg	CPU-II_2 100 μmol/kg	Colchicine 6 μmol/kg	OLA 50 μmol/kg
Body weight (g)	40.7 ± 1.5	38.3 ± 1.7	38.2 ± 1.0	39.2 ± 1.4	36.2 ± 1.0	38.1 ± 1.4
Liver weight (g)	2.2 ± 0.11	2.0 ± 0.12	2.1 ± 0.18	2.0 ± 0.1	1.7 ± 0.1**	1.7 ± 0.1**
ALT (U/L)	81.98 ± 8.10	714.8 ± 46.9##	525.6 ± 48.6	451.9 ± 14.1**	455.6 ± 44.7**	694 ± 49.9
AST (U/L)	68.0 ± 15.4	790.7 ± 67.3##	526.3 ± 37.4*	476.8 ± 38.4**	401.1 ± 39.0**	661.4 ± 57.3
LDH (U/L)	2175.5 ± 197.1	4636 ± 362.8##	4008.5 ± 187.3	3682.8 ± 142.0*	3755.7 ± 203.6	4148 ± 220.1
Hydroxyproline (mg/g liver)	117.0 ± 9.4	363.7 ± 51.3##	338.7 ± 91.6	263.1 ± 55.2**	229.4 ± 49.0**	343.9 ± 87.1

Data are expressed as the mean ± SEM. The number of mice in each column is 8. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ compared with Normal group; $^{*}P < 0.05$, $^{**}P < 0.01$ compared with Control group.

as liver Hyp content compared with normal mice. Against the control mice that were treated with CCl_4 and given saline, the administration of CPU-II_2 significantly reduced the blood ALT and AST levels elevated by CCl_4. At the dose of 100 μmol/kg, CPU-II_2 also significantly decreased serum LDH and liver Hyp levels. However, the mother compound OLA at 50 μmol/kg only showed a slight tendency to reduce the ALT and AST activities. Colchicine also significantly decreased ALT, AST and Hyp. In this case, CPU-II_2 did not reduce body weights and liver weights of the mice, while colchicine significantly decreased the liver weights and tendentiously reduced the body weights.

Pathological examination of the mice liver sections indicated that CPU-II_2 remarkably ameliorated adipose degeneration of hepatocytes, but did not affect the inflammatory cell infiltration (Fig. 2 H.E.). As shown in Fig. 2A, as stained by Masson, the collagen fibers in CCl_4-treated mice were obviously more than those in normal mice. When treated with CPU-II_2, the collagen deposition in liver was decreased by the dose of 50 μmol/kg, and the collagen fibers nearly disappeared by 100 μmol/kg.

Collagen content was also determined by measurement of Hyp content assay in the livers (Table 1). The mean Hyp level was significantly higher in the CCl_4-treated group ($P < 0.01$).

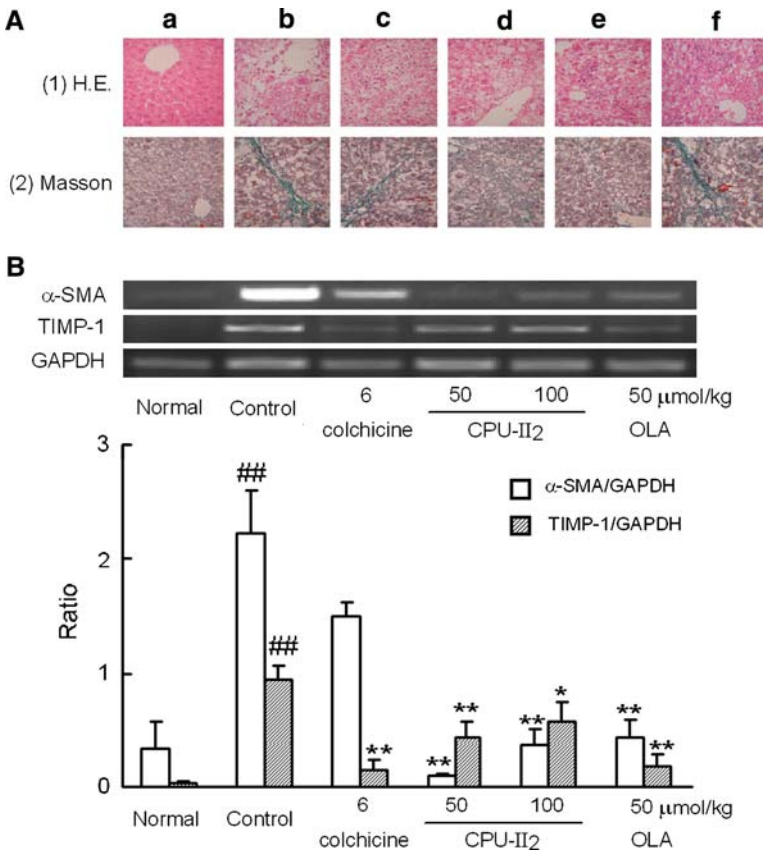

Figure 2. Histopathological changes in CCl_4-induced liver fibrosis mice receiving CPU-II_2 treatment. (**A**) The sections of mice liver were stained with (1) hematoxylin-eosin or (2) Masson and examined by a blinded histologist. (**a**) from normal mouse; (**b**) from control mouse; (**c**) from mouse treated with 50 μmol/kg of CPU-II_2; (**d**) from mouse treated with 100 μmol/kg of CPU-II_2; (**e**) from mouse treated with 6 μmol/kg of Colchicine; (**f**) from mouse treated with 50 μmol/kg of OLA. (Original magnification ×200). (**B**) Analysis for gene transcripts of α-SMA and TIMP-1 genes in liver tissues. The total mRNA was extracted from liver tissue of each mouse, then the mRNA expressions of α-SMA and TIMP-1 were detected by RT-PCR. Top: sample RT-PCR. Bottom: histograms of RT-PCR analyses. Intensities of α-SMA and TIMP-1 bands were respectively normalized to that of GAPDH of corresponding treatment groups. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ compared with Normal group; $^{*}P < 0.05$, $^{**}P < 0.01$ compared with Control group. The data are representative of three independent experiments.

CPU-II_2 at 100 μmol/kg significantly reduced the value of liver Hyp. Colchicine also decreased liver Hyp significantly.

There were significantly increased expressions in liver α-SMA and TIMP-1 mRNA in CCl_4-treated mice compared with those in normal mice (Fig. 2B). Against the increased expressions, CPU-II_2, OLA and colchicine showed a marked inhibition.

Effects of CPU-II_2 on cell viability of HSC-T6 cells

CPU-II_2 neither influenced the viability of HSC-T6 cells after treatments (Fig. 3), nor induced the apoptosis of HSC-T6 cells at 24 h after treatments (data not shown), while colchicine showed a significant inhibition on the viability of HSC-T6 cells (Fig. 3).

Effects of CPU-II_2 on α-SMA expression in HSC-T6 cells

HSC-T6 cells were activated HSCs characterized by high expression of α-SMA [4, 5]. The CPU-II_2 treatment dose-dependently reduced both mRNA and protein expressions of α-SMA in HSC-T6 cells (Fig. 4).

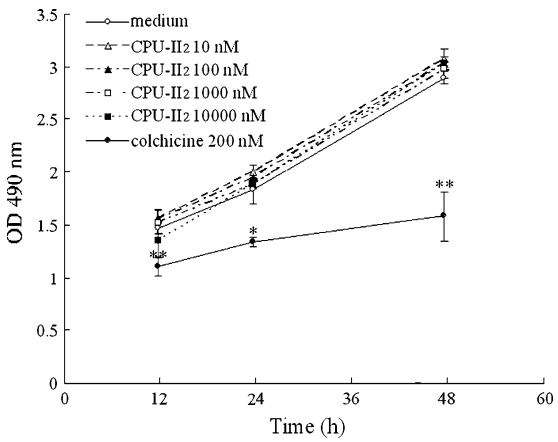

Figure 3. Effect of CPU-II_2 on cell viability of HSC-T6 after treatments. After HSC-T6 cells were treated with CPU-II_2 of different concentrations or colchicine (200 nM) for 12, 24 and 48 h, the CytoTox 96 assay was carried out to detect the cell number as described in the Materials and Methods. Data are given in the form of mean ± SEM of three experiments and each experiment includes triplicate wells. $*P < 0.05$, $**P < 0.01$ compared with medium group.

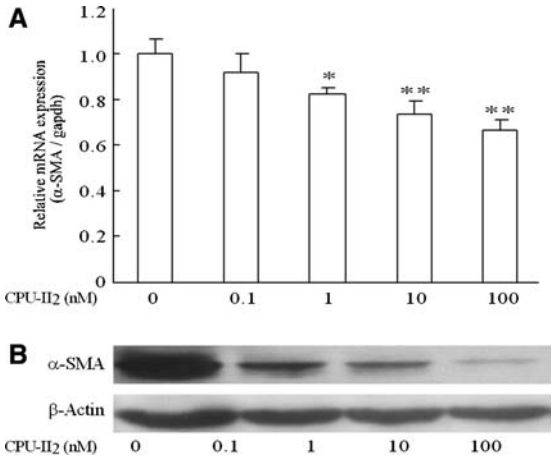

Figure 4. Effects of CPU-II_2 on mRNA and protein expressions of α-SMA in HSC-T6 cells. (**A**) Quantitative real-time polymerase chain reaction analysis for the expression of α-SMA in HSC-T6 cells at 24 h after treatments. Data are expressed as the mean ± SEM of three independent experiments. $*P < 0.05$, $**P < 0.01$. (**B**) expression levels of α-SMA protein detected by western blot at 48 h after treatments. Representative data from three individual experiments are depicted here.

Effect of CPU-II_2 on the mRNA expression of procollagen-α1 (I) in HSC-T6 cells

The expression of procollagen-α1 (I) mRNA on HSC-T6 cells was measured by quantitative PCR. As shown in Fig. 5, CPU-II_2 dose-dependently decreased the mRNA expression of pro-collagen I in HSC-T6 cells.

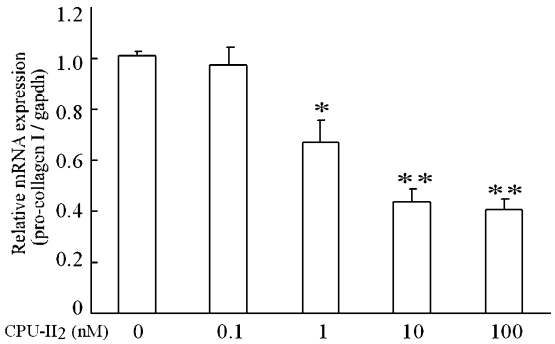

Figure 5. Quantitative real-time PCR analysis for the expression of EMC-related gene procollagen-α1 (I). Data are expressed as the mean ± SEM of three independent experiments. $*P < 0.05$, $**P < 0.01$.

Effect of CPU-II$_2$ on the expression of phospho-p38 MAPK protein in HSC-T6 cells and LX-2 cells

As revealed by Western blot analysis, pretreatment with 0.01–100 nM CPU-II$_2$ inhibited the expression of phospho-p38 MAPK (Fig. 6A). LX-2 (human HSC line) cells were also treated with 1–100 nM CPU-II$_2$ in the presence of 2 ng/ml TGF-β1 for 48 hours. This compound also reduced the expression of phosphorylated p38 (Fig. 6B).

Discussion

The effective anti-fibrotic drugs are required for the treatment of liver fibrosis, which should be liver-specific, low toxic and effective in attenuating excessive collagen deposition. However, the current drugs such as corticosteroids and colchicine, usually show various side effects due to immune suppression or cytotoxicity [22, 23]. Due to the physiological roles of quiescent HSCs in the regulation of ECM remodeling [7], the drug for liver fibrosis by targeting the HSCs seems to be focused on the activation or function of HSCs rather than on the cell viability. In fact, inhibition of the accumulation of activated HSCs and prevention of the deposition of ECM have been linked to the resolution for liver fibrosis [2].

The present study has documented that CPU-II$_2$ significantly attenuated the experimental hepatic fibrosis induced by CCl_4 in mice and exerted inhibitory effects on HSC-T6 cells. The Hyp content, collagen deposition, and mRNA expression of α-SMA and TIMP-1 genes in the liver of CCl_4-treated mice with high dose (100 μmol/kg) of CPU-II$_2$ were significantly reduced in comparison with those of CCl_4-treated mice receiving saline, together with the improvement of hepatic injury markers including plasma AST, ALT and LDH activities. However, the mother compound of CPU-II$_2$, OLA only showed a slight tendency to reduce the ALT, AST, LDH and Hyp contents. This finding suggests that the structure modification for OLA produces a possibility for OLA derivatives to become a novel candidate for anti-fibrotic drugs.

On the other hand, we observed that CPU-II$_2$ decreased the expression of TIMP-1 in vivo, which was known to strongly promote the liver fibrosis development [24], suggesting that the prevention of liver fibrosis by CPU-II$_2$ is related to the inhibition of TIMP-1.

In addition, α-SMA has been known as a specific activation marker of HSCs [5], and the HSC is the predominant cell type responsible for excess collagen deposition during liver fibrosis [25]. The reduction in the expression of α-SMA in vivo by CPU-II$_2$ suggests that this compound may influence the activation of HSCs. However, this compound neither affected the cell viability nor induced the apoptosis of HSC-T6 cells in vitro. For confirming whether CPU-II$_2$ affects the stellate cells, we designed other assays and found that the compound dose-dependently decreased levels of the gene transcription and protein expression of

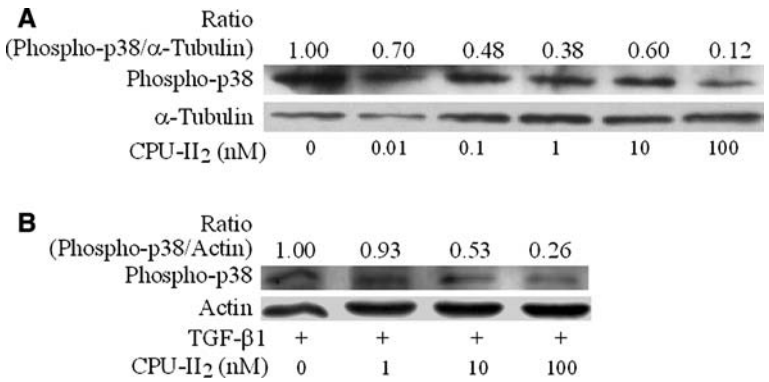

Figure 6. Effect of CPU-II$_2$ on the phosphorylation of p38 MAPK. (**A**) HSC-T6 cells were treated with CPU-II$_2$ for 48 h. The signal intensity of the Phospho-p38 bands was normalized to that of the corresponding α-tubulin protein. (**B**) LX-2 cells were treated with CPU-II$_2$ for 48 h in the presence of TGF-β1(2 ng/ml). The signal intensity of the Phospho-p38 bands was normalized to that of the corresponding Actin protein. The untreated CPU-II$_2$ group was assigned a value of 1. Representative data from three individual experiments are depicted here.

α-SMA, as well as the mRNA expression of procollagen-α1 (I) in HSC-T6 cells. These data suggest that CPU-II_2 rather reduces the function of HSC-T6 cells than causes the cell death. This characteristic may be unique and beneficial to the long term treatment of liver fibrosis, which is different from gliotoxin that is known to induced HSC apoptosis [26, 27]. These results also consist with and reconfirm our findings from previous studies in vivo. Thus, CPU-II_2 seemed to persuade activated HSCs to become quiescent-like HSCs. OLA also reduced mRNA expression of α-SMA and TIMP-1 (Fig. 2B). However, Liver fibrosis appears multiple stages and is regulated by many factors such as MMPs, TIMPs, TGF-β and platelet-derived growth factor (PDGF). In addition to α-SMA and TIMP-1, other markers should also be emphasized for the evaluation of antifibrotic drugs, such as collagen deposition and MAPKs activation. Indeed, in the present study, CPU-II_2 also showed a marked regulation on collagen deposition while OLA did not.

Moreover, the p38 MAPK signaling pathway is involved in the induction of procollagen-α1(I) mRNA by TGF-β1 in rat glomerular mesangial cells [28]. The p38 MAPK signaling independently and additively regulates alpha1(I) collagen gene expression by transcriptional activation, and increases alpha1(I) collagen mRNA stability [29] and p38 MAP kinase also mediates PDGF-BB-stimulated hepatic myofibroblasts migration, but not proliferation [30]. Dilinoleoylphosphatidylcholine (DLPC), the active component of polyenylphosphatidylcholine extracted from soybeans, decreased TGF-β1-induced collagen mRNA by inhibiting p38 MAPK in HSCs [31]. These findings suggest that the p38 MAPK serves as a key molecule in HSCs to secrete alpha1 (I) collagen. In this study, we found that CPU-II_2 could also decrease the phospho-p38 MAPK matching with the reduction of pro-collagen I in HSC-T6 cells and we also found that CPU-II_2 could decrease the phospho-p38 MAPK in LX-2 cells. The antifibrotic effect of CPU-II_2 via down regulating p38 MAPK may be a new character for liver fibrosis resolution.

In conclusion, we demonstrated that CPU-II_2 inhibited CCl_4-induced liver fibrosis, and its antifibrosis effects may be related to the inhibition of the function of activated HSCs through regulating p38 MAPK pathways without damaging the cell itself. A possible clinical application due to this unique character could be expected to have benefits for chronic liver disorders accompanied by liver fibrosis.

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