JPP Journal of Pharmacy and Pharmacology

JPP 2002, 54: 119–126

Received May 14, 2001
Accepted July 24, 2001
ISSN 0022-3573

Curcumin inhibits collagen synthesis and hepatic stellate cell activation in-vivo and in-vitro

Hee-Chul Kang, Ji-Xing Nan, Pil-Hoon Park, Ji-Young Kim, Sung Hee Lee, Sun Wook Woo, Yu-Zhe Zhao, Eun-Jeon Park, and Dong Hwan Sohn

Abstract

We previously demonstrated that curcumin, a well-known antioxidant, inhibits collagen deposition in carbon tetrachloride-induced liver injury in rats. The major effector cells responsible for collagen synthesis in the liver are activated hepatic stellate cells. In this study, we investigated the inhibitory effects of curcumin on the collagen synthesis and activation of rat hepatic stellate cells in-vitro, and on hepatic stellate cell activation in-vivo. The effects of curcumin on the production of collagen and smooth muscle α-actin proteins and of α1(I) collagen mRNA were studied in-vivo and in-vitro. The effect of curcumin on DNA synthesis was also determined in-vitro. In-vivo, treatment with curcumin reduced collagen deposition and smooth muscle α-actin-positive areas and lowered mRNA levels of type I collagen in the liver. In-vitro, curcumin at a concentration of 5 μg mL^{-1} reduced DNA synthesis, and downregulated smooth muscle α-actin and type I collagen expression, and α1(I) collagen mRNA expression. We concluded that curcumin inhibits collagen synthesis and hepatic stellate cell activation in-vivo and in-vitro, and thus may prove a valuable anti-fibrogenic agent.

Department of Pharmacy, Medicinal Resources Research Center, Wonkwang University, Iksan, Jeonbuk 570-749, South Korea

Hee-Chul Kang, Ji-Xing Nan, Pil-Hoon Park, Ji-Young Kim, Sung Hee Lee, Sun Wook Woo, Yu-Zhe Zhao, Eun-Jeon Park, Dong Hwan Sohn

College of Pharmacy, Yanbian University, Yanjim, Jilin, 133000, People's Republic of China

Ji-Xing Nan

Correspondence: Dong Hwan Sohn, Department of Pharmacy, Medicinal Resources Research Center, Wonkwang University, Iksan, Jeonbuk 570-749, South Korea. E-mail: dhsohn@wonkwang.ac.kr

Funding: This research was supported by a grant (# PF002105-01) from the Plant Diversity Research Center of the 21st Frontier Research Program funded by the Ministry of Science and Technology of the Korean government and by a Wonkwang University Grant for Professor Dong Hwan Sohn (2001).

Introduction

The natural polyphenolic compound curcumin, known to be a strong antioxidant, occurs in the rhizomes of *Curcuma longa* Linnaeus (turmeric: Ammon & Walh 1991). It has been reported to have anti-inflammatory (Ammon & Walh 1991), anti-carcinogenic (Rao et al 1995), liver-protective (Akila et al 1998; Chuang et al 2000; Park et al 2000a), antioxidant (Ruby et al 1995) and pulmonary-protective (Punithavathi et al 2000; Venkatesan 2000) effects. It also inhibits the accumulation of collagen in the tail tendons and skin of diabetic rats (Sajithlal et al 1998).

The structural organization of the liver reflects its function as a guardian between the digestive tract and the rest of the body (Miyai 1991). Because of this interposition, it receives large amounts of noxious substances, as well as nutrients entering the body through the digestive tract and hepatic portal vein. Hence, the liver is vulnerable to damage by many agents (Miyai 1991). The effect of hepatic injury depends on the extent of damage induced by the particular agent. If the injury is mild, the liver cells will recover completely, restoring liver function to normal. Fortunately, this is the usual outcome. If the injury is severe and chronic, large amounts of liver tissue may be destroyed. Healing of this severe injury may be associated with severe scarring, and liver functions may never return to normal. Any such chronic or progressive liver injury may cause scarring and functional impairment, resulting in liver fibrosis or cirrhosis.

During chronic liver damage, hepatic stellate cells (HSCs) undergo a response known as 'activation', which is the transition of quiescent cells into proliferative, fibrogenic and contractile myofibroblasts expressing collagen and smooth muscle α-actin (α-SMA), resulting in liver fibrosis (Friedman et al 1985; Knittel et al 1992; Friedman 2000). Agents that inhibit HSC activation either in-vivo or in-vitro might therefore be candidates for the therapeutic prevention of chronic liver injury and/or liver fibrosis (Friedman 1993).

We previously demonstrated that curcumin reduces the serum levels of alanine transaminase and aspartate transaminase, and reduces hydroxyproline and lipid peroxide contents in cases of liver injury induced by carbon tetrachloride (Park et al 2000a). Some other studies have also demonstrated that turmeric and/or curcumin inhibits liver injury (Kiso et al 1983; Donatus et al 1990; Akila et al 1998). However, direct evidence to support its effect on HSCs during liver injury in-vitro or in-vivo is not yet available. In this study, the effects of curcumin on collagen synthesis and HSC activation were investigated in damaged rat livers and in isolated HSCs activated in-vitro by determining DNA synthesis, the expressions of collagen and α-SMA genes based on protein levels, and the levels of mRNA for α1(I) procollagen.

Materials and Methods

In-vivo study

Induction of experimental liver injury

Male Sprague–Dawley rats (160–180 g) were purchased from Dae Han Laboratory Animal Research and Co. (Choongbuk, Korea) and were allowed free access to the normal standard chow diet (Jae Il Chow, Korea) and tap water. The rats were housed four or five per cage and were kept under controlled conditions of 22±2°C, 50–60% relative humidity and 12-h light–dark cycles (from 7:00 to 19:00) throughout the experiments. The rats were maintained in these facilities for 1 week before the experiment.

Carbon tetrachloride was given orally (1 mL kg^{-1}, mixed with an equal volume of corn oil) on Mondays and Thursdays for 4 weeks to induce chronic liver injury (Park et al 2000a). During this treatment, curcumin (Sigma Chemical Co., St Louis, MO) was suspended in corn oil and administered to the rats daily (100 mg per 2 mL kg^{-1} body weight/day, orally). Experimental groups are shown in Table 1. Three days after the last carbon tetrachloride treatment, the rats were killed under ether anaesthesia. Liver samples were removed and immediately snap frozen in liquid nitrogen. They were kept at −70°C until assayed.

Table 1 Hydroxyproline levels in the livers of carbon tetrachloride-intoxicated rats treated with curcumin.

Group	n	Hydroxyproline (μg g^{-1} liver)
Normal	6	294±43
Vehicle-treated	6	312±42
Curcumin+vehicle	6	325±53
Vehicle+CCl_4	8	723±243*
Curcumin+CCl_4	8	405±102**

Results are shown as means±s.d. Normal: untreated normal rats. Vehicle-treated: rats orally treated with corn oil (daily; vehicle for curcumin) and corn oil (twice a week; vehicle for CCl_4) simultaneously. Curcumin+vehicle: rats orally treated with curcumin (100 mg kg^{-1} day^{-1}) and corn oil, twice a week simultaneously. Vehicle+CCl_4: rats orally treated with corn oil (daily) and carbon tetrachloride (1 mL kg^{-1}) simultaneously. Curcumin+CCl_4: rats orally treated with curcumin (100 mg kg^{-1} day^{-1}) and carbon tetrachloride (1 mL kg^{-1}) simultaneously. *Significantly different from normal, $P < 0.001$. **Significantly different from rats treated with vehicle+CCl_4, $P < 0.01$.

Histology and immunohistochemistry of injured livers

A portion of liver samples was fixed by immersion in 10% neutral formalin (pH 7.4) for 24 h. The fixed tissue was then dehydrated in a graded series of ethanol, embedded in paraffin and sectioned at a thickness of 4 μm. Sections were stained using Masson's trichrome method for the detection of connective tissue deposition. For the detection of activated-hepatic stellate cells in fibrotic livers, α-SMA was assessed immunohistochemically by the streptavidin–biotin–peroxidase complex method using a LSAB 2 kit (DAKO Co., Carpinteria, USA) and monoclonal mouse anti-α-SMA antibody (Roche Molecular Biochemicals, Mannheim, Germany).

Image analysis of α-SMA expression in damaged liver

Histomorphometric analysis was performed using a VISUS image processor equipped with a microscopy digital camera (Sound Vision SVMicro), which provides digital images at a resolution of 960×800 pixels. The analyser can store 24-bit colour images. Numerous mathematical morphological functions were available for analysis in a compatible Pentium-based personal computer. The mounted liver sections were placed on the stage of a Leitz DIAPLAN microscope after equali-

zation of light intensity. A numeric image of the complete section was stored in the colour image processor at a final magnification of ×10.

The stained areas were calculated automatically. For the morphometric analysis of α-SMA expression in damaged liver, the relative liver area expressing α-SMA was calculated based on the mean percentage of three sections, and was defined as the area expressing α-SMA. The scores of five separate samples from each group were then averaged.

Liver hydroxyproline content

Collagen concentrations in liver were estimated by measuring hydroxyproline concentrations (Jamall et al 1981).

In-vitro study

Isolation and cultivation of HSCs

Hepatic stellate cells were isolated from the livers of untreated normal male Sprague–Dawley rats weighing 450–500 g by two-step collagenase perfusion (Park et al 2000b). Isolated cells were more than 90% viable as assessed by trypan blue exclusion, and consisted of more than 85% hepatic stellate cells as determined by immunostaining with horseradish peroxidase coupled anti-desmin (Sigma).

Measurement of DNA synthesis

DNA synthesis was analysed by bromodeoxyuridine (BrdU) incorporation using an ELISA kit (Roche Molecular Biochemicals). To investigate BrdU incorporation, activated HSCs, cultured on plastic dishes for 7 days after isolation, were plated at a density of 1×10^4 cells/well in 96-well plates and grown to confluence in William's Medium E (WME, Gibco BRL, USA) containing 10% fetal bovine serum (FBS, GibcoBRL) under standard culture conditions. The confluent cells were growth-arrested in serum-free medium for 48 h. Curcumin was dissolved in dimethylsulfoxide (DMSO) with a final concentration in the medium of 1%. After growth arrest, cells were incubated with 50 ng mL^{-1} platelet-derived growth factor-BB (PDGF-BB; Pinzani et al 1989) and either vehicle (controls) or a given concentration of curcumin (0.1, 1 and 5 μg mL^{-1}). They were then labelled with BrdU for 24 h. After removing the culture medium, the cells were fixed, incubated with anti-BrdU POD and the incorporated BrdU was detected by the subsequent substrate reaction according to the manufacturer's instructions.

HSC viability

HSC viability during curcumin treatment was assessed by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT; Sigma) assay (Park et al 2000b).

Western blot analysis for α-SMA in-vitro

Western blotting for α-SMA in HSCs was processed as described by Park et al (2000b). HSCs were incubated with either vehicle or a final concentration of curcumin (1, 2.5, 5 μg mL^{-1} for an additional 8 days. Monoclonal mouse anti-α-SMA (Sigma; diluted 1:1000) was used as a primary antibody, and alkaline phosphatase conjugated goat anti-mouse IgG (Sigma; diluted 1:250) was the secondary antibody.

Western blot analysis for type I collagen in-vitro

Activated HSCs, initially cultured on plastic dishes for 7 days after isolation, were plated at a density of 5×10^5 cells in 60 mm culture dishes and grown to confluence in WME containing 10% FBS under standard tissue culture conditions. Subsequently, cells were incubated with either vehicle (DMSO) or curcumin (1, 2.5 and 5 μg mL^{-1} final concentrations) for 2 days in the presence of 0.1 mM ascorbic acid (Sigma) and 0.5 mM β-aminopropionitrile (Sigma) in serum-free medium. After 48 h, the medium was collected and the protein was precipitated with 10% trichloroacetic acid. The pellet was solubilized in 0.1 M NaOH. The remaining HSCs were lysed in Ripa buffer. Aliquots of 20 μg of protein were used for electrophoresis and immunoblotting. Polyclonal rabbit anti-collagen type I (Calbiochem, La Jolla, USA) was used as a primary antibody, and goat anti-rabbit IgG conjugated to phosphatase (Sigma) as the secondary antibody.

RNA isolation and Northern blot analysis

To clone rat α1(I) collagen, reverse-transcription polymerase chain reaction (RT-PCR) was employed on samples of bile duct ligation and scission operation induced rat cirrhotic livers. Rat α1(I) collagen-specific primers were synthesized according to the following nucleotide sequences: rat type I collagen, sense primer 5′TCC GTG ACC GTG ACC TTG AG 3′and antisense primer 5′ CTT GGG GTT TGG GCT GAT GT 3′. The PCR products were cloned into Topo Vector (Invitrogen, Carlsbad, USA), sequenced by an automated DNA sequencer (AB310; Perkin-Elmer, Japan) and used as cDNA probes.

Total RNA was isolated from liver samples kept at −70°C and curcumin-treated HSCs using an RNeasy

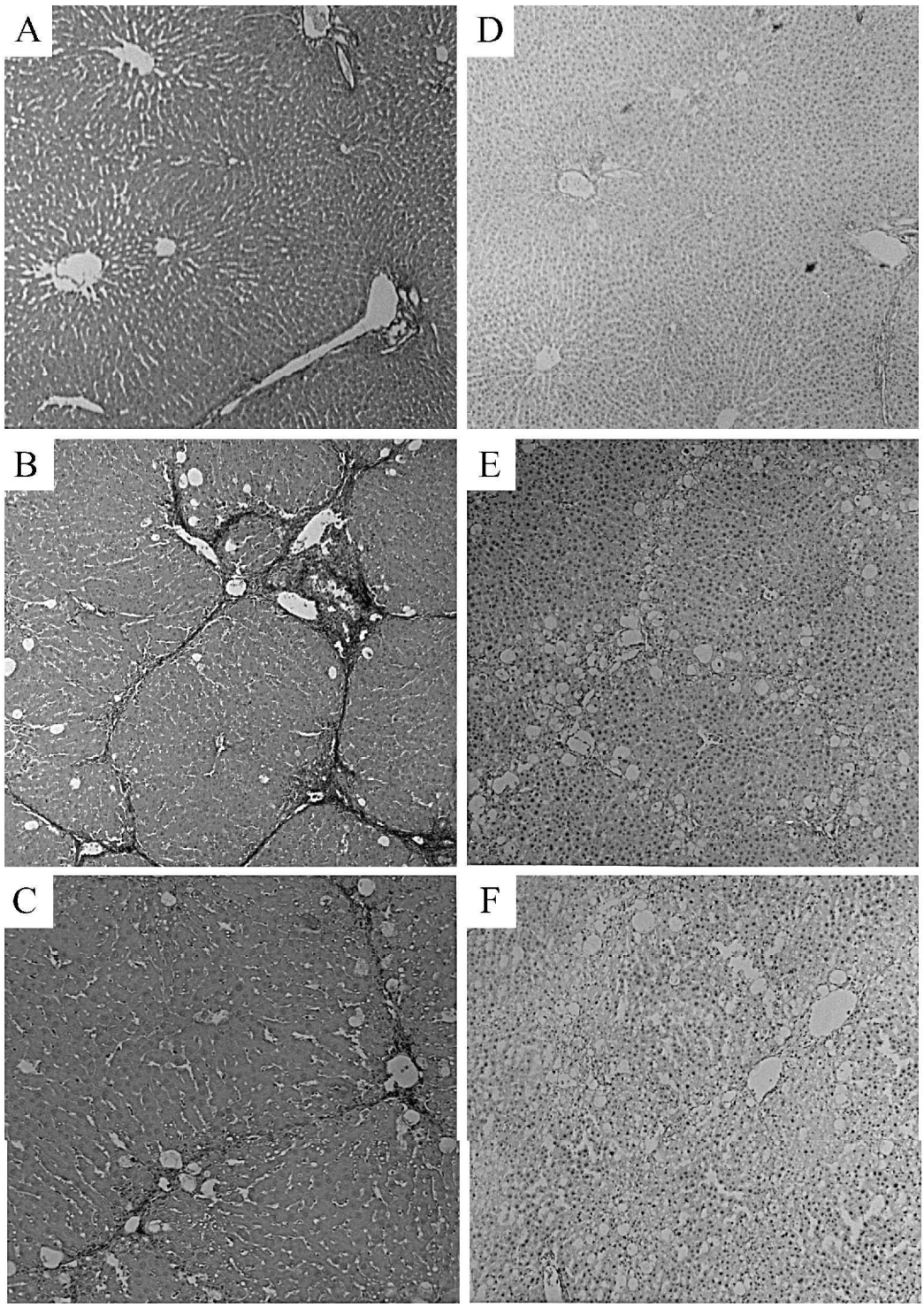

Figure 1 Masson's trichrome staining for collagen and immunohistochemical appearance for α-SMA of representative liver sections treated with curcumin. Carbon tetrachloride was orally administered (1 mL kg^{-1}) twice a week for 4 weeks. Curcumin (100 mg kg^{-1} day^{-1}) was administered orally to rats daily during carbon tetrachloride treatment (original magnification $\times 100$). Normal rat liver: panel A, Masson's trichrome; panel D, α-SMA immunostaining. Rat liver treated with vehicle and carbon tetrachloride: panel B, Masson's trichrome; panel E, α-SMA immunostaining. Rat liver treated with curcumin and carbon tetrachloride: panel C, Masson's trichrome; panel F, α-SMA immunostaining.

Miniprep kit (Quiagen Inc., Valencia, USA). Ten micrograms of total RNA was separated by 1% agarose gel containing 5.4% formaldehyde electrophoresis, transferred to nylon membrane (NEN, Boston, MA) and hybridized with specific [α-^{32}P] dCTP-labelled cDNA probes. cDNA probes were labelled by random priming kit (Promega, Madison, WI). Hybridization was carried out at 55°C. Post-hybridization washes were performed once for 10 min at room temperature and for 30 min at 55°C in 1 × SSC containing 5% sodium dodecyl sulfate. Nylon membranes were autoradiographed using Kodak X-ray film, using intensifying screens at −70°C.

Statistical analysis of the results

All values are expressed as means ± s.d. When groups of more than three were compared, one-way ANOVA and Turkey's multiple comparison tests were performed. Comparisons between vehicle-treated carbon tetrachloride rat liver and curcumin-treated carbon tetrachloride rat liver in quantitative morphometric analysis were performed using Student's *t*-test. Statistically significant differences between groups were defined as P values of less than 0.05. Calculations were performed with the GraphPad Prism program (GraphPad Software, Inc., San Diego, USA).

Ethical considerations

This experiment was carried out under the Guiding Principles in the Use of Animals in Toxicology adopted by the Society of Toxicology (USA) in July 1989 and revised in March 1999. The animal care committee in our institution approved this study.

Results

In-vivo study

Histochemical detection of collagen and α-SMA expression

As shown in Figure 1, carbon tetrachloride treatment for 4 weeks resulted in liver injury, with loss of normal lobular architecture and a marked increase of collagen deposition throughout the liver (Figure 1B). However, curcumin treatment resulted in reduced collagen deposition in the liver of carbon tetrachloride-treated rats (Figure 1C). In normal livers, collagen was observed only around the central and portal veins (Figure 1A).

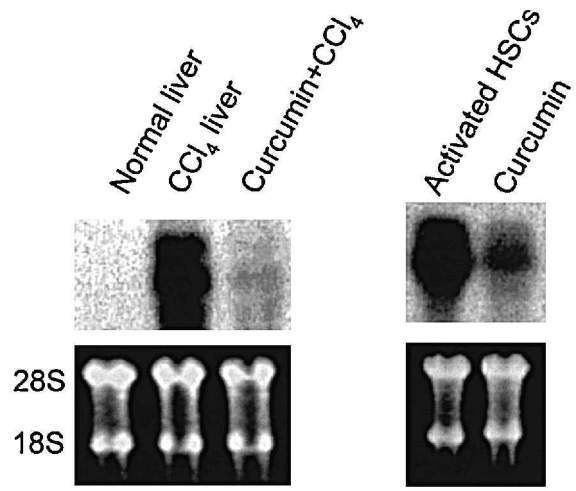

Figure 2 Northern blot analysis of α1(I) collagen mRNA expression in damaged rat liver treated with curcumin (upper left panel) or in culture-activated hepatic stellate cells incubated with curcumin (upper right panel). Total RNA was purified from the liver of rats treated orally with curcumin (100 mg^{-1} kg^{-1} day) and carbon tetrachloride (1 mL kg^{-1} twice a week) for 4 weeks. Hepatic stellate cells were isolated from normal rat liver, cultured on plastic dishes for 7 days, and then incubated for 2 days with vehicle or 5 μg mL^{-1} of curcumin. Samples of 10 μg of RNA were used. Lower panels (left panel: rat liver; right panel: HSCs) show RNA formaldehyde agarose gel comparing the amount of 28S and 18S rRNA of the different samples. The graphs represent typical results of three independent experiments.

HSC activation was examined immunohistochemically by determining α-SMA-positive areas in injured rat livers treated with curcumin. Considerable expression was detected in areas of centrilobular and periportal fibrotic bands in carbon tetrachloride- plus vehicle-treated rats (Figure 1E). In contrast, curcumin notably reduced the positive areas of α-SMA in the livers of rats treated with carbon tetrachloride (Figure 1F). Quantitative morphometric analysis of these livers, stained against α-SMA, demonstrated that curcumin significantly decreased the area of HSC activation from 2.5 ± 0.7% in damaged livers to 1.0 ± 0.1% in curcumin-treated damaged livers ($P < 0.001$). In normal rat livers (Figure 1D), α-SMA-positive areas were rarely observed.

Hydroxyproline content in curcumin-treated liver

Liver hydroxyproline content was increased to 246% in carbon tetrachloride-treated rats ($P < 0.01$) when compared with normal control rats. In rats treated with 100 mg kg^{-1} curcumin, hydroxyproline content was

decreased to 56% of that of carbon tetrachloride-treated control rats ($P < 0.001$; Table 1).

Expression of α1(I) collagen mRNA in curcumin-treated liver

The effects of curcumin on α1(I) collagen mRNA expression was investigated by Northern blotting analysis using 10 μg of total RNA extracted from the livers of rats treated with carbon tetrachloride plus vehicle, or carbon tetrachloride plus curcumin (Figure 2). Expression of α1(I) collagen mRNA levels was markedly increased in the damaged livers treated with carbon tetrachloride, as compared with control normal livers treated with vehicle alone. However, the mRNA expression level of α1(I) collagen was notably decreased in the livers treated with carbon tetrachloride plus curcumin when compared with that in livers treated with carbon tetrachloride plus vehicle (Figure 2). A representative formaldehyde agarose gel comparing the amounts of 28S and 18S rRNA of the different samples is shown in Figure 2.

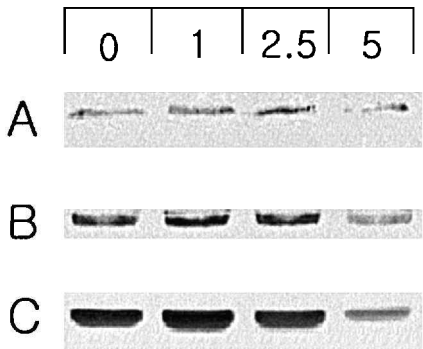

Figure 4 Western blot analysis for type I collagen and smooth muscle α-actin expression in activated rat hepatic stellate cells treated with curcumin at concentrations of 0, 1, 2.5 and 5 μg mL^{-1}. For measurement of type 1 collagen, hepatic stellate cells, isolated from normal rat liver and cultured on plastic dishes for 7 days, were incubated for 2 days with a given concentration of curcumin. For the measurement of smooth muscle α-actin, hepatic stellate cells, isolated from normal rat liver and cultured on plastic dishes for 2 days, were incubated for 8 days with a given concentration of curcumin. (A) Type I collagen secreted into culture medium. (B) Type I collagen in cell lysates. (C) Smooth muscle α-actin in cell lysates. The graphs show typical results of three independent experiments.

In-vitro study

DNA synthesis in curcumin-treated HSCs

As shown in Figure 3, curcumin reduced BrdU incorporation in a dose-dependent manner in activated HSCs stimulated with 50 ng mL^{-1} PDGF-BB. BrdU incorporation in PDGF-BB-stimulated HSCs was 164±15% of that of unstimulated HSCs, while it was significantly reduced to 115±8% ($P < 0.05$) and 72±11% ($P < 0.001$) in 1 μg mL^{-1} and 5 μg mL^{-1} curcumin-treated PDGF-BB-stimulated HSCs, respectively (Figure 3). To eliminate the possibility that curcumin might be toxic for HSCs, cell viability was assessed by MTT assay. This showed that curcumin did not alter the cell viability in concentrations used in the experiment (data not shown).

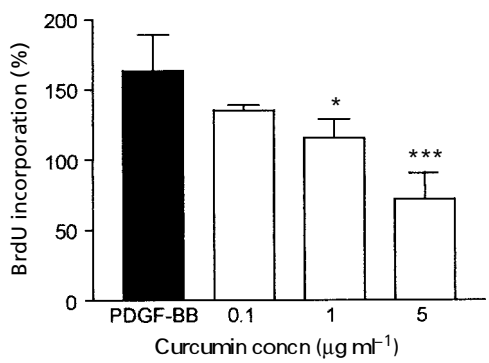

Figure 3 Effects of curcumin on DNA synthesis of culture-activated hepatic stellate cells stimulated with PDGF-BB. BrdU incorporation in cells was determined after incubation for 24 h with 50 ng mL^{-1} PDGF-BB and curcumin at concentrations of 0, 0.1, 1 and 5 μg mL^{-1}. The control value without PDGF-BB was 100% (not shown). Results are the means±s.d. of three independent experiments, each performed using triplicate wells. *Significantly different from HSC treated with 50 ng mL^{-1} PDGF-BB alone ($P < 0.05$). ***Significantly different from HSC treated with 50 ng mL^{-1} PDGF-BB alone ($P < 0.001$).

Type I collagen and α-SMA expression in curcumin-treated HSCs

The inhibitory effects of curcumin on type I collagen and α-SMA gene expression in activated HSCs cultured on plastic dishes were investigated by measuring protein levels using immunoblotting. Two days of curcumin treatment at a concentration of 5 μg mL^{-1} decreased type I collagen to about 52% and 54% in culture medium (Figure 4A) and in HSC lysates (Figure 4B), respectively, when compared with activated HSCs treated with vehicle only. The expression of α-SMA was also reduced to about 57% in HSCs cultured with curcumin at a concentration of 5 μg mL^{-1} for 8 days (Figure 4C). However, curcumin at concentrations of 1 μg mL^{-1} or 2.5 μg mL^{-1} had little or no effect on α-SMA or type I collagen expression in HSCs. The MTT assay showed that cell viability was not altered in the conditions used in the experiment (data not shown).

Production of α1(I) collagen mRNA in curcumin-treated HSCs

HSCs, isolated from normal rat liver, were initially cultured for 7 days in plastic dishes for full activation, and then treated with curcumin at a concentration of 5 μg mL^{-1} for 2 days before α1(I) collagen and TGF-β1 mRNA assays. Curcumin at a concentration of 5 μg mL^{-1} decreased α1(I) collagen mRNA levels in activated HSCs when compared with activated HSCs without curcumin treatment (Figure 2, right panel). RNA integrity and loading amount was qualified by 1% agarose gel electrophoresis, as shown in the lower right panel in Figure 2.

Discussion

The main hallmark of chronic liver disease is the accumulation of α-SMA-positive HSCs within the expanding fibrous septa or in the perisinusoidal spaces (Rockey et al 1992). HSCs are the major cell type responsible for collagen synthesis in chronic liver injury (Friedman 1993). These cells display a quiescent phenotype in the normal liver and acquire fibroblastic features following acute or chronic liver injury. HSCs showing fibroblastic features are called activated HSCs. During chronic liver injury, activated HSCs proliferate and synthesize most extracellular matrix components, which accumulate in damaged liver, resulting in liver fibrosis. Given the pivotal role of activated HSCs in chronic liver injury, agents that could regulate HSC activation might be promising candidates for therapeutic agents for collagen deposition in chronic liver injury. Our earlier report revealed that curcumin reduces liver hydroxyproline content, indicating that it inhibits collagen deposition in carbon tetrachloride-induced liver injury (Park et al 2000a). In the present study, histochemical analysis on Masson's trichrome-stained sections demonstrated that curcumin significantly decreased collagen deposition, confirming the previous report. Immunohistochemistry revealed that α-SMA-positive cells decreased in number after treatment with curcumin at a dose of 100 mg kg^{-1} day^{-1} for 4 weeks during carbon tetrachloride administration, suggesting that curcumin inhibits HSC activation and proliferation during liver injury in-vivo. Curcumin also downregulated collagen mRNA synthesis during liver injury in-vivo. These in-vivo results led us to conduct in-vitro experiments to find out if curcumin could act directly on HSC activation and proliferation. HSC proliferation, a crucial step in the development of liver fibrosis, was measured by BrdU incorporation. PDGF-BB, a most potent proliferative cytokine (Pinzani et al 1989), was used to stimulate HSC proliferation. PDGF dimers are reported to play a pivotal role in mediating the proliferation of HSCs. Accordingly, inhibitors that block PDGF-stimulated HSC proliferation may provide potential new therapeutic strategies for the treatment of collagen deposition during chronic liver injury. The in-vitro results showed that curcumin significantly inhibited the proliferation of activated HSCs. This inhibitory effect on cell proliferation was consistent with other studies using different cell types (Singh et al 1996; Gautam et al 1998; Chen et al 1999). Curcumin inhibited the expression of α-SMA, the phenotypic marker of activated HSCs, in a dose-dependent manner, which shows that curcumin inhibits HSC activation. Additionally, curcumin reduced collagen type I at both the protein and expressed mRNA levels. From in-vivo and in-vitro studies, it is evident that curcumin attenuates HSC activation and proliferation and reduces the production of collagen at protein and mRNA levels.

Conclusions

Curcumin reduced the accumulation of α-SMA-positive areas in this model system, indicating that curcumin inhibits HSC activation in chronic liver injury induced by carbon tetrachloride in rats. Although its mechanism is not known, it is evident that curcumin decreased collagen and α-SMA expressions at protein levels, and type I collagen at mRNA level, and these are upregulated in activated HSCs in-vitro. Curcumin also attenuated DNA synthesis in activated HSCs. From these findings, we believe that curcumin may be a useful candidate for developing therapeutics for the prevention and treatment of collagen deposition in cases of chronic liver injury.

References

Akila, G., Rajakrishnan, V., Viswanathan, P., Rajashekaran, K. N., Venugopal, P. (1998) Effects of curcumin on lipid profile and lipid peroxidation status in experimental hepatic fibrosis. *Hepatol. Res.* **11**: 147–157

Ammon, H. P., Wahl, M. A. (1991) Pharmacology of *Curcuma longa*. *Planta Med.* **57**: 1–7

Chen, H., Zhang, Z. S., Zhang, Y. L., Zhou, D. Y. (1999) Curcumin inhibits cell proliferation by interfering with the cell cycle and inducing apoptosis in colon carcinoma cells. *Anticancer Res.* **19**: 3675–3680

Chuang, S., Cheng, A., Lin, J., Kuo, M. (2000) Inhibition by curcumin of diethylnitrosamine-induced hepatic hyperplasia, inflammation, cellular gene products and cell-cycle-related proteins in rats. *Food Chem. Toxicol.* **38**: 991–995

Donatus, I. A., Sardjoko, Vermeulen, N. P. (1990) Cytotoxic and cytoprotective activities of curcumin: effects on paracetamol-induced cytotoxicity, lipid peroxidation and glutathione depletion in rat hepatocytes. *Biochem. Pharmacol.* **39**: 1869–1875

Friedman, S. (1993) The cellular basis of hepatic fibrosis : mechanisms and treatment strategies. *N. Engl. J. Med.* **328**: 1828–1835

Friedman, S. L. (2000) Molecular regulation of hepatic fibrosis, an integrated cellular response to tissue injury. *J. Biol. Chem.* **275**: 2247–2250

Friedman, S. L., Roll, F. J., Boyles, J., Bissell, D. M. (1985) Hepatic lipocytes: the principal collagen-producing cells of normal liver. *Proc. Natl. Acad. Sci. USA* **82**: 8681–8685

Gautam, S. C., Xu, Y. X., Pindolia, K. R., Janakiraman, N., Chapman, R. A. (1998) Nonselective inhibition of proliferation of transformed and nontransformed cells by the anticancer agent curcumin (diferuloylmethane). *Biochem. Pharmacol.* **55**: 1333–1337

Jamall, I. S., Finelli, V. N., Que Hee, S. S. (1981) A simple method to determine nanogram levels of 4-hydroxyproline in biological tissues. *Anal. Biochem.* **112**: 70–75

Kiso, Y., Suzuki, Y., Watanabe, N., Oshima, Y., Hikino, H. (1983) Antihepatotoxic principles of *Curcuma longa* rhizomes. *Planta Med.* **49**: 185–187

Knittel, T., Schuppan, D., Meyer zum Buschenfelde, K. H., Ramadori, G. (1992) Differential expression of collagen types I, III, IV by fat-storing (Ito) cells in vitro. *Gastroenterology* **102**: 1724–1735

Miyai, K. (1991) Structural organization of the liver. In: Meeks, R. G., Harrison, S. D., Bull, R. J. (eds) *Hepatotoxicology.* CRC Press, Boca Raton, FL, pp 1–65

Park, E. J., Jeon, C. H., Ko, G. I., Kim, J. B., Sohn, D. H. (2000a) Protective effect of curcumin in rat liver injury induced by carbon tetrachloride. *J. Pharm. Pharmacol.* **52**: 437–440

Park, P. H., Nan, J. X., Park, E. J., Kang, H. C., Kim, J. Y., Ko, G., Sohn, D. H. (2000b) Effect of tetrandrine on experimental hepatic fibrosis induced by bile duct ligation and scission in rats. *Pharmacol. Appl. Toxicol.* **87**: 261–268

Pinzani, M., Gesualdo, L., Sabbah, G. M., Abboud, H. E. (1989) Effects of platelet-derived growth factor and other polypeptide mitogens on DNA synthesis and growth of cultured rat liver fat-storing cells. *J. Clin. Invest.* **84**: 1786–1793

Punithavathi, D., Venkatesan, N., Babu, M. (2000) Curcumin inhibition of bleomycin-induced pulmonary fibrosis in rats. *Br. J. Pharmacol.* **131**: 169–172

Rao, C. V., Rivenson, A., Simi, B., Reddy, B. S. (1995) Chemoprevention of colon carcinogenesis by dietary curcumin. A naturally occurring plant phenolic compound. *Cancer Res.* **55**: 259–266

Rockey, D. C., Boyles, J. K., Gabbiani, G., Friedman, S. L. (1992) Rat hepatic lipocytes express smooth muscle actin upon activation in vivo and in culture. *J. Submicrosc. Cytol. Pathol.* **24**: 193–203

Ruby, A. J., Kuttan, G., Babu, K. D., Rajasekharan, K. N., Kuttan, R. (1995) Anti-tumor and antioxidant activity of natural curcuminoids. *Cancer Lett.* **94**: 79–83

Sajithlal, G. B., Chithra, P., Chandrakasan, G. (1998) Effect of curcumin on the advanced glycation and cross-linking of collagen in diabetic rats. *Biochem. Pharmacol.* **56**: 1607–1614

Singh, A. K., Sidhu, G. S., Deepa, T., Maheshwari, R. K. (1996) Curcumin inhibits the proliferation and cell cycle progression of human umbilical vein endothelial cell. *Cancer Lett.* **107**: 109–115

Venkatesan, N. (2000) Pulmonary protective effects of curcumin against paraquat toxicity. *Life Sci.* **66**: PL21–28