BASIC STUDIES

Prevention of liver cirrhosis in rats by curcumin

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Keywords
cirrhosis – curcumin – hepatic stellate cells – thioacetamide

Abbreviations
ROS, reactive oxygen species; NFκB, nuclear factor kappa B; TAA, Thioacetamide; TNF-α, tumor necrosis factor alpha; TGF, transforming growth factor; HSC, hepatic stellate cells; MMP, matrix metalloproteinase.

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Abstract
Background and Aim: Curcumin, the major polyphenolic compound in turmeric, has been shown to attenuate hepatic damage in several animal models of liver injury. The aim of the present study was to examine the efficacy of curcumin in preventing thioacetamide-induced cirrhosis and to unravel the mechanism of curcumin’s effect on hepatic fibrosis in rats. Methods: Liver cirrhosis was induced by thioacetamide (TAA; 200 mg/kg, i.p.) twice weekly for 12 weeks. One group of rats concomitantly received curcumin (300 mg/kg/day, by gavage for 12 weeks); the control group received the solvent at identical amounts and duration. Results: TAA administration induced liver cirrhosis, which was inhibited by curcumin. Liver histopathology, hydroxyproline levels and spleen weights were significantly lower in the rats treated with TAA + curcumin compared with TAA only (P < 0.001). Immunohistochemical studies and in situ hybridization demonstrated inhibition of hepatic stellate cell (α smooth muscle actin-positive) activation and collagen α1(I) gene expression in the livers of the TAA + curcumin-treated rats. Curcumin reduced oxidative stress as shown by the decreased hepatic nitrotyrosine staining in the curcumin + TAA-treated rats. Curcumin treatment had no effect on pre-existing liver cirrhosis. As determined by in vitro studies using the rat HSC-T6 cell line, curcumin had no direct inhibitory effect on collagen α1(I) messenger RNA expression. Further studies in these cells using reverse transcriptase-polymerase chain reaction demonstrated that curcumin had no effect on the expression of PDGF-induced TIMP-1 and TIMP-2, TGFβ1, TGFβ2 and MCP-1 but significantly inhibited tumor necrosis factor alpha expression. Curcumin had no effect on hepatic stellate cells proliferation. Zymography showed that curcumin had no effect on matrix metalloproteinase-2 activity. Conclusion(s): Curcumin inhibited the development of TAA-induced liver cirrhosis mainly due to its anti-inflammatory activities and not by a direct anti-fibrotic effect. As curcumin ingestion is safe in humans, it may be reasonable to assess in clinical studies the beneficial effect of curcumin in slowing the development of liver cirrhosis.

Certain forms of hepatic injury lead to a chronic, partially self-perpetuating inflammation and an attempt at tissue regeneration and wound healing progressing in some patients to liver cirrhosis. Chronic tissue injury and inflammation lead to activation of hepatic stellate cells (HSC), which become contractile and deposit collagen in the space of Disse. This can eventually lead to disruption of the functional structure of hepatic lobules and to increased resistance to portal blood flow (1). Increased collagen deposition between hepatocytes and sinusoids and the diminution of the size of endothelial fenestrae lead to the capillarization of sinusoids. Constriction of sinusoids by contractile HSC and capillarization increase resistance to blood flow and contribute to the development of portal hypertension (1, 2). Complications of portal...
hypertension are the main causes of mortality in patients with cirrhosis (2). Although progress has been made in the management of liver cirrhosis and its complications, preventive strategies should be beneficial in reducing the burden of this disease (1, 3). The rhizome of turmeric (Curcuma longa), a plant belonging to the ginger family, is widely used as a food colouring and is one of the principal ingredients in curry powder. Turmeric has long been used in Indo-Chinese medicine as an anti-inflammatory to treat digestive and liver disorders, and for the treatment of skin diseases and wound healing (4). The main active ingredient in turmeric is curcumin (diferuloylmethane) (4), administration of which is therapeutic in rodent models of a number of intestinal, pancreatic and liver diseases (5–7). Anti-inflammatory, antioxidant and nuclear factor-κB (NF-κB) inhibiting properties may underlie curcumin's beneficial effect in these conditions (8). It has been reported that curcumin prevents collagen type I formation by activated HSC (9) and reduces hepatic fibrosis and inflammation in rodent models of steatohepatitis (10, 11).

Curcumin ingestion is safe in humans, and is sufficiently bioavailable to produce beneficial systemic and hepatic effects (12). Utilizing the chronic administration of thioacetamide (TAA) to rats as a model, our aim in this study is to unravel the mechanism of curcumin's effect on hepatic fibrosis: is it a direct effect on HSC and collagen formation or an indirect effect by the prevention of inflammation and necrosis

Materials and methods

Materials

TAA, curcumin, cremophore and glycerol formal were obtained from Sigma Chemicals Co. (St. Louis, MO). Curcumin was dissolved in glycerol formal, cremophore and water (5:2:2).

A 1600-bp rat collagen α1 (I) probe was a generous gift from B.E.Kream, University of Connecticut, Farmington, CT. Smooth muscle actin monoclonal antibodies, used at 1:1200 dilution, were obtained from Dako A/S (Glostrup, Denmark). As a second antibody, the Histomouse SP kit was used (Zymed Laboratories Inc, South San Francisco, CA).

Animals

Male Wistar rats (250–300 g), obtained from Tel Aviv University Animal Breeding Center, were fed a Purina chow ad libitum. Animals were kept with a 12 h light–dark cycle at constant temperature and humidity, and the rats had free access to tap water during the study period. Animals received humane care and were treated according to institutional guidelines.

Experimental design

Prevention of cirrhosis

For the induction of liver cirrhosis, rats were given intraperitoneal (i. p.) injections of TAA, 200 mg/kg, twice a week for 12 weeks, as previously described (13). Two groups of six rats each were exposed to (a) bi-weekly i.p. TAA (200 mg/kg) and daily i.g. solvent administration for 12 weeks; (b) bi-weekly i.p. TAA (200 mg/kg) and daily intrragingival curcumin (300 mg/kg, dissolved in solvent as described above and given in a final volume of 2 ml per rat) administration for 12 weeks. A third group received only the solvent and served as control.

Treatment of cirrhosis

This experiment was performed to determine whether curcumin would have a beneficial effect on established liver cirrhosis. Such an effect may support a mechanism that includes direct anti-fibrotic or fibrinolytic effects. Five groups of rats were studied (a) TAA 12 weeks, (b) and (c) TAA 12 weeks followed for another 4 or 6 weeks by 300 mg/kg/day curcumin given by gavage after TAA discontinuation, (d) and (e) TAA 12 weeks followed by solvent administration (2 ml/day) for 4 or 6 weeks after TAA discontinuation.

At the end of the study, the rats were sacrificed, their livers were removed and the spleen weights were measured.

Analysis of liver histopathology

Midsections of the left lobes of the livers were processed for light microscopy. This processing consisted of fixing the specimens in a 5% neutral formol solution, embedding the specimens in paraffin, making 5 μm thick sections and staining the sections with hematoxylin & eosin. The tissue slices were scanned and scored blindly by two experts. The degree of fibrosis, classified on a scale of 0–3 according to Muller et al. (14), was expressed as the mean of 10 different fields in each slide. Pathological alterations consistent with fibrosis, nodule formation and the presence of fibrotic septa were included in the fibrosis score (0–3). Inflammatory infiltration, hepatocyte apoptosis and breaking up of the hepatocellular limiting plates in the portal tracts consisted of the inflammation score (0–3).
Measurement of hepatic hydroxyproline levels

Quantitative determination of hepatic hydroxyproline content was performed as previously described (15).

Assessment of hepatic nitrotyrosine

Nitrotyrosine, the product of protein oxidation, was assessed in paraffin blocks of the different treatment groups. Sections were stained with mouse antinitrotyrosine (Zymed Laboratories, San Francisco, CA) at 1:250 dilution for 1 h. As second antibodies, we used Universal HRP-polymer kit (BioCare Medical, Concord, CA) according to the manufacturer instructions.

Preparation of sections, in situ hybridization and immunohistochemistry

Liver samples were collected into phosphate-buffered saline (PBS) and fixed overnight in 4% paraformaldehyde in PBS at 4 °C. Serial 5 μm sections were prepared after the samples had been dehydrated in graded ethanol solutions, cleared in chloroform and embedded in Paraplast. Differential staining of collagenous and non-collagenous proteins was performed with 0.1% Sirius red and 0.1% fast green as a counter stain, in saturated picric acid. By this procedure, collagen is stained red (16). For hybridization, the sections were deparaffinized in xylene, rehydrated through graded ethanol solutions, and air dried for several hours. Before hybridization, the 1600 bp rat collagen α1(I) insert was cut out from the original plasmid (pUC18) and inserted into Safyre. The sections were then hybridized with digoxigenin-labeled collagen α1(I) probe (17). For immunohistochemistry, smooth muscle actin antibodies were used, and the detection was performed with the Histomouse SP kit according to the manufacturer’s instructions.

Effect of curcumin on collagen α1(I) gene expression

SV40-immortalized rat HSC-T6 cell line (18), generously provided by Dr. S. L. Friedman (Mount Sinai Medical Center, NY), were incubated with curcumin (5 or 10 μg/ml) for 24 h. Collagen α1(I) gene expression was determined by Northern blot analysis using the collagen α1(I) probe.

Northern blots and probes

Total RNA from HSC-T6 cell line was isolated with TRI reagent (Molecular Research Center Inc., Cincinnati, OH) according to the manufacturer’s instructions. For Northern blots, 10 μg of total RNA was resolved under denaturing conditions on 1.5% agarose/formaldehyde gel and transferred onto Nytran N nylon membranes by capillary transfer. The membranes were baked at 80 °C for 2 h and prehybridized at 68 °C for 2 h with EZ-hybridization buffer. Following prehybridization, 32P-labeled cDNA probe were added into the hybridization solution and hybridized with the membranes at 68 °C overnight. Membranes were then washed twice with 2 × SSC 0.1% sodium dodecyl sulphate (SDS) at 42 °C for 15 min and twice with 0.1 × SSC/0.1% SDS at 65 °C for 30 min and for 1.5 h. Autoradiography was performed on Kodak X-ray films with intensifying screens at −70 °C. The probes used for the Northern blot hybridization were generated by reverse transcriptase-polymerase chain reaction (RT-PCR) amplification using the following primers pairs for human collagen type I: 5′-AGCAGAAAACATCGGATTG-3′, 5′-GAGGAGGGGTTCAGAGGAG-3′. The 18S and 28S ribosomal RNA were used as control for the quantity of the RNA loaded in each lane.

Cell culture conditions

The immortalized rat hepatic stellate cell line HSC-T6 were grown in Dulbecco’s modified Eagle medium (DMEM; Biological Industries, Bet-Haemek, Israel) supplemented with 10% fetal calf serum (FCS) (v/v), 2 mM L-glutamine, and 100 mg/ml of penicillin–streptomycin. 24 h later the medium was changed to hormonally defined medium (HDM). HDM consisted of DMEM supplemented with 100 mg/ml penicillin–streptomycin, 2 mg/ml bovine serum albumin, 610 mg/ml nicotinamide, 740 ng/ml ZnSO4.7H2O, 20 ng/ml CuSO4.5H2O, 5 mM glutamine, 5 mg/ml insulin, 5 mg/ml iron-saturated transferrin, 5 mg/ml selenous acid and 10−7 M dexamethasone (all materials were obtained from Sigma).

Proliferation assay

Rat HSC were incubated in a 24-well plate (30 000 cells/well). The cells grew in DMEM culture medium supplemented with 10% FCS until the logarithmic growth phase, and then incubated for 48 h in HDM supplemented with or without curcumin (0.5,
1, 5, 10 or 15 μM). After 48 h, cells were trypsinized and counted using hemocytometer.

**Gelatin zymography assay**

Rat HSC were plated on six-well (80 000 cells per well). After 24 h in DMEM 10% FCS media was replaced with HDM supplemented with or without 5 μM curcumin. After 48 h the supernatant was collected and cell number was evaluated using hemocytometer. The supernatant was mixed with nonreducing sample buffer (0.4 M Tris, pH. 6.8%, 5% SDS, 20% glycerol, 0.03% bromophenol blue). Proteins were separated by electrophoresis through 8% polyacrylamide gel-containing gelatin (25mg/ml). Following electrophoresis, gels were washed in 2.5% Triton X-100 with gentle shaking for 30 min, and then incubated for 30 min in developing buffer (40 mM Tris-HCl, 0.2 mM NaCl, 6.67 mM CaCl2, 0.1% Triton X-100, pH. 7.8). The developing buffer was replaced with fresh buffer and the gel incubated overnight at 37°C. The gels were stained with 0.5% Coomasie blue G-250 in 10% acetic acid and 40% methanol and destained in water. MMP-2 activity was visualized as bands of lysis, which appear white on dark background.

**RNA extraction and RT-PCR**

Total RNA was extracted from rat hepatic stellate cell line (HSC-T6) using the EZ-RNA kit. Its quantity and quality was assessed by measuring the optical density at 260 and 280 nm. cDNA was synthesized from total RNA using the M-MLV Reverse Transcriptase kit (Promega, Madison, WI, USA), according to manufacturer’s instructions.

The cDNAs were amplified by PCR using REDTaq DNA polymerase. Primers were designed to detect TNF-α sense 5’-ATGAGCACAGAAGCATGATC-3’, antisense 5’-CAGAGCAATGACTCCAAAGTA-3’, TGF-β1 (sense 5’-TGGCGTTACCTTGGTAACC-3’, antisense 5’-GTTGTGGAGGCCTTTCCAG-3’), TGF-β2 (sense 5’-ATCGATGGCACCTCCACATATG-3’, antisense 5’-ACCACAGTCATGCAATGACTCCAAAGTA-3’), TIMP-1 (sense 5’-TGGCGTTACCTTGGTAACC-3’, antisense 5’-TCTA-CAGAAGTGCGTTAGGATGTGG-3’), m-RNA expression levels were normalized to the expression of glyceraldehyde-3-phosphatedehydrogenase (GAPDH) (sense 5’-ACCACAGTCATGCAATGACTCCAAAGTA-3’). The conditions for amplification were 5 min at 94°C, 30 cycles of 30 s at 94°C, 30 s at 56°C and 30 s at 72°C, followed by extension for 5 min at 72°C. PCR products were resolved by electrophoresis through 1% agarose gels.

**Statistical analysis**

Results of in vivo studies are presented as the mean ± SD. The significance of differences among different groups was determined by ANOVA and followed by a post hoc test.

The data of the in vitro studies are presented as mean ± SE. Experiments were replicated at least three times. Statistical analysis was performed with unpaired two-tailed Student’s t-test. P-values less than 0.05 were considered significant.

**Results**

**Liver histopathology**

Histopathologic examination of liver specimens, fixed after 12 weeks of treatment, showed significantly less nodule formation, fibrotic septa (Table 1 and Fig. 1, P < 0.001), inflammatory infiltration and hepatocyte necrosis and apoptosis (Fig. 2, P < 0.01) in the livers of TAA+curcumin-treated rats relative to TAA only.

**Quantitative analysis of hydroxyproline**

Liver fibrosis was quantitated by the measurement of hepatic levels of hydroxyproline. The mean hydroxyproline levels of the TAA-treated group were significantly higher than those of both groups of rats co-treated with curcumin (11.7 ± 3.1 vs. 4.4 ± 0.6, P < 0.001, Table 1).

Table 1. Effect of curcumin on prevention of TAA-induced liver cirrhosis

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Hydroxyproline (mg/g protein)</th>
<th>Spleen weight (g)</th>
<th>Fibrosis score (0–3)*</th>
<th>Inflammation (0–3)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAA + vehicle</td>
<td>11.7 ± 3.1</td>
<td>1590 ± 123</td>
<td>2.7 ± 0.7</td>
<td>2.2 ± 0.7</td>
</tr>
<tr>
<td>TAA + curcumin</td>
<td>4.4 ± 0.6*</td>
<td>970 ± 118*</td>
<td>0.5 ± 0.3*</td>
<td>0.4 ± 0.3*</td>
</tr>
<tr>
<td>Vehicle only</td>
<td>1.8 ± 0.5*</td>
<td>870 ± 126*</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Means ± SD, n = 6 in each group.

*A P < 0.001 compared with TAA only. TAA, thioacetamide, 200 mg/kg i.p. twice weekly for 12 weeks. Curcumin, 300 mg/kg, five times/week.

†A scale of 0–3: no change, 0; slight changes, 1; stronger changes, 2; and intense changes, 3.
Spleen weight

Characteristic hemodynamic changes of liver cirrhosis have previously been shown after 3 months of TAA administration, including portal hypertension and hyperdynamic circulation that are accompanied by a significant increase in spleen weight (13). In our study, after 12 weeks of treatment, the mean spleen weight of rats receiving TAA only was 39% lower compared with those receiving TAA + curcumin (\(P < 0.001\), Table 1).

Effect of curcumin on the regression of pre-established cirrhosis

This experiment was performed to determine whether curcumin would have a beneficial effect on established liver cirrhosis. Such an effect may support a mechanism that includes direct anti-fibrotic or fibrinolytic effects. As shown in Table 2, curcumin administration for 4 or 6 weeks to rats that already received TAA for 12 weeks had no effect on liver histology, hepatic hydroxyproline levels or the spleen weights, indicating that curcumin has no effect on pre-established liver cirrhosis.

Hepatic content of nitrotyrosine

As shown in Fig. 3, nitrotyrosine staining was increased in the livers of the rats treated with TAA for 12 weeks and significantly reduced in the livers of those treated concomitantly with curcumin. This confirms that curcumin treatment reduced oxidative stress in the livers of the TAA-treated rats, and that this effect could be observed not only after acute TAA administration, as shown in our previous studies (19), but also after prolonged treatment with TAA that causes liver cirrhosis.

Activation of HSC (\(\alpha\) smooth muscle actin) and collagen \(\alpha 1\) (I) gene expression in vivo

Liver sections of the control rats were devoid of collagen. In the control livers, when stained with \(\alpha\) smooth muscle actin antibodies, no stellate cells were detected, suggesting that the latter were in their quiescent state. No cells expressing the collagen \(\alpha 1(1)\) gene were detected by in situ hybridization (Fig. 1) or immunohistochemistry (not shown). When treated with TAA, the livers exhibited a marked increase in extracellular matrix (ECM) content and displayed bundles of collagen surrounding the lobules, which resulted in large fibrous septa and distorted tissue architecture. These septa were populated by smooth muscle actin-positive cells expressing high levels of the collagen \(\alpha 1(1)\) gene, all of which are characteristic of advanced fibrosis and cirrhosis. Cotreatment with curcumin prevented the activation of most of the HSC and only traces of smooth muscle-positive cells were detected. The remaining HSC expressed low levels of collagen \(\alpha 1(1)\) gene that resulted in low levels of collagen as determined by Sirius red staining (Fig. 1).
Effect of curcumin on collagen $\alpha_1$(I) gene expression in hepatic stellate cells

Incubation of HSC-T6 cells with curcumin (5 or 10 $\mu$g/ml) for 24 h did not decrease collagen $\alpha_1$(I) gene expression as determined by Northern blot analysis using the collagen $\alpha_1$(I) probe (Fig. 4).

Effect of curcumin on cytokine expression in HSC in vitro

We assessed whether curcumin influenced cytokine expression in HSC-T6 cells. Total m-RNA was extracted from HSC-T6 cells 24 h following incubation in HDM supplemented with or without 5 $\mu$M curcumin. We have shown using RT-PCR that curcumin significantly inhibited HSC expression of TNF-$\alpha$, but not that of TIMP-1, TIMP-2, TGF$\beta$1, TGF$\beta$2 and MCP-1 in HSC-T6 cells following stimulation by platelet-derived growth factor (PDGF) (Fig. 5). Curcumin did not cause any significant change in cytokine expression in unstimulated cells and had no effect on HSC proliferation (Fig. 6).

Effect of curcumin on MMP-2 activity in HSC-T6 cells

We examined the effect of curcumin on MMP-2 activity using gelatine zymography assay. Cultured hepatic stellate cells were incubated in HDM media in the presence or absence of 5 $\mu$M curcumin for 24 h. There was no difference in MMP-2 activity in HSC-T6 cells in the presence of curcumin (Fig. 7).
Discussion

TAA, although not toxic itself, is metabolized into potent hepatotoxins by hepatic cytochromes (20). These can produce liver injury by the formation of highly reactive compounds (21, 22) and possibly also by activating NFκB (23). The form of hepatic damage produced by TAA depends on the dosage and duration of its administration: high doses can cause fulminant hepatic failure (FHF) and early death (24), whereas lower dose administered over a prolonged period of time culminates in liver cirrhosis (17). We recently demonstrated that pre-treatment with curcumin provided hepatoprotection in a rodent model of TAA-induced FHF (19). In that study, curcumin inhibited lipoperoxidation and activated NFκB and iNOS expression in the liver. Survival rates were higher in curcumin-treated rats (19). Curcumin does not

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**Fig. 3.** Effect of curcumin on hepatic nitrotyrosine. In the control, untreated rats very low level of nitrotyrosine was observed. Livers of rats treated with TAA exhibited a high number of stellate cells of which many exhibited nitrotyrosine. After curcumin treatment a marked decrease in nitrotyrosine content was observed.
significantly alter the activity of hepatic CYP2E1 (25), the major isoform involved in the formation of toxic TAA metabolites (26, 27). Its beneficial effects in the present and latter study can therefore be attributed to the amelioration of TAA metabolites’ toxicity, rather than their formation. The objective of the present study was to assess whether dietary curcumin could attenuate the development of full-blown cirrhosis and portal hypertension (manifested as splenomegaly) in rats due to chronic TAA administration, and to further explore the mechanisms responsible for curcumin’s anti-fibrotic effect. Rats that received bi-weekly, i.p. injections of TAA for 12 weeks developed hepatic...

Fig. 4. Effect of curcumin on collagen α1(I) gene expression in HSC in vitro. Incubation of HSC-T6 cells with curcumin (5 or 10 μg/ml = 15 or 30 μM) for 24 h had no effect on collagen α1(I) gene expression as determined by Northern blot analysis using the collagen α1(I) probe. The 18S and 28S ribosomal RNA were used as control for the quantity of the RNA loaded in each lane.

Fig. 5. (A) Curcumin inhibits TNFα expression by stimulated HSC-T6 cell line. Total RNA was extracted from rat hepatic stellate cell line (HSC-T6) incubated in hormonally defined medium supplemented with 25 ng/ml platelet-derived growth factor with or without curcumin (5 or 15 μM). cDNA was synthesized from ~2 μg total RNA and amplified using primers designed to detect TNF-α, TGF-β1, TGF-β2, TIMP-1, TIMP-2 and MCP-1. mRNA expression levels were normalized to the expression of GAPDH. Results represent the expression level in curcumin treated cells compared with the expression level in untreated cells. (B) Quantitation of the bands corresponding to the effect of curcumin on cytokine expression in HSC in vitro was performed by densitometry. Results represent the expression level in curcumin-treated cells compared with the expression level in untreated cells. The graph shows representative results from at least 3 independent experiments (means ± SE, *P value < 0.05).
cirrhosis, manifested by gross macroscopic appearance, liver histopathology, hepatic hydroxyproline content and increased spleen weight. Their livers also robustly exhibited the primary components of HSC activation, i.e. increased expression of \( \alpha \) smooth muscle actin and type I collagen gene. In these \textit{in vivo} studies, these indices and mediators of fibrosis and cirrhosis were significantly lower in the rats that were administered curcumin concomitantly with TAA. In fact, the pathological changes were virtually prevented by curcumin.

In \textit{in vitro} studies, collagen \( \alpha_1(I) \) mRNA levels were not influenced by incubation with curcumin. The inhibition of collagen \( \alpha_1(I) \) expression shown \textit{in vivo} probably represents an effect on early events leading to activation of collagen \( \alpha_1(I) \) expression such as necroinflammation and oxidative stress. We also showed using RT-PCR that curcumin inhibited HSC expression of TNF-\( \alpha \), but not that of TIMP-1 TIMP-2, TGF\( \beta_1 \), TGF\( \beta_2 \) and MCP-1 in unstimulated state and following stimulation with PDGF, and had no effect on HSC proliferations. Thus, curcumin did not inhibit HSC proliferation or expression of profibrogenic genes, despite its NF\( \kappa B \)-inhibitory properties (10). Failure of targeted NF\( \kappa B \) inhibition to prevent phenotype-related gene expression or induce apoptosis in activated HSC has previously been reported (28).

Why does the anti-inflammatory effects of curcumin inhibit hepatic fibrogenesis? A complex interplay between resident and infiltrating cell types takes place during the development of fibrosis and cirrhosis of the liver, but activation of HSC is invariably the final common pathway (29, 30). The metabolically active hepatocytes are the main target of toxins such as TAA and carbon tetrachloride (30, 31) that lead to hepatocyte dysfunction and release of reactive oxygen species (ROS), inflammatory and fibrogenic mediators, as well as to necrosis and apoptosis. Circulating white blood cells are recruited and activated, and, in cohort with Kupffer cells secrete growth factors that activate HSC, as well as other components of the pro-inflammatory and fibrogenic cascade. In addition, apoptotic hepatocytes activate HSC, and also upregulate the inflammatory state apart from increasing the production of ECM proteins. Thus, a vicious cycle in which inflammatory and fibrogenic cells stimulate each other is likely to occurs (1–3, 29–31).

Strategies for reducing the burden of cirrhosis include removal of the injurious stimulus, limiting hepatic injury and inflammation, and inhibition/reversal of HSC activation and fibrosis (1). For instance, agents that reduce oxidative stress and inhibit the activation of NF\( \kappa B \) are effective in attenuating TAA-induced cirrhosis (21–23). Although not assessed in this study of a chronic model, curcumin does minimize \textit{in vivo} liver NF-\( \kappa B \) activation and oxidative stress in acute TAA toxicity (19), as well as in rodent models.
of steatohepatitis (10, 11). It has been shown that pretreatment with curcumin inhibited NF-κB binding activity and TNF-α expression in Kupffer cells exposed to endotoxin, as well as in vivo in livers of rats-fed with toxic doses of ethanol and fish oil (10). These gene products promote inflammation, tissue injury and HSC activation (1, 29–32).

To what can we attribute curcumin’s protective effect against hepatic cirrhosis in our study? It is reasonable to suggest that inhibition of NFκB in Kupffer and recruited inflammatory cells in curcumin-treated rats may have contributed to the reduced tissue injury and activation of HSC. We have shown in a previous study using a model of acute TAA-hepatotoxicity that curcumin inhibited the formation of hepatic lipo-peroxides in rats acutely treated with TAA, as evident by reduced TBARS level, indicating an attenuation of oxidative stress (25). Curcumin also reduced hepatic lipoperoxide formation in both acute and chronic carbon tetrachloride injury (6, 33). It has been suggested that curcumin inhibits formation of ROS and enhances endogenous anti-oxidant activity beyond its free radical scavenging property (8). In the liver, curcumin induces and/or activates a number of hepatic antioxidant enzymes, including catalase, superoxide dismutase and the glutathione system (8, 34, 35). As ROS and lipoperoxides are potent inducers of HSC activation (30), curcumin’s anti-oxidative properties may have attenuated HSC-mediated fibrogenesis in this study. Therefore, it appears that its protective role in chronic TAA administration is in virtue of its anti-inflammatory and possibly its anti-oxidative capacity. By attenuating the vicious cycle of cell injury and production of ROS, chemokines, cytokines and profibrotic molecules, curcumin may both protect hepatocytes from demise and dysfunction (19), and also prevent the formation of the molecules involved in transforming normally quiescent HSC into a proliferative, fibrogenic and contractile myofibroblast. The resultant development of cirrhosis and portal hypertension is thus prevented, as clearly shown in this study. This is supported by the attenuation of oxidative stress after acute TAA administration, as shown by decreased malondialdehyde (MDA) formation in the curcumin-treated rats after 48 h in our previous studies (19). In the present study of chronic TAA administration we used nitrotyrosine, a product of protein oxidation, and observed a marked decrease in nitrotyrosine staining in the hepatocytes and HSC of the TAA+curcumin-treated rats compared with TAA only. These results confirm that the inhibition of oxidative stress in the liver by curcumin could be demonstrated not only after acute TAA administration as shown previously (19), but also after prolonged treatment with TAA that causes liver cirrhosis. Hence, curcumin’s protective effect against liver fibrosis may be associated with its ability to inhibit NF-κB in Kupffer cells and infiltrating macrophages, and thus prevent hepatic inflammation, necrosis and apoptosis. This is represented by the significant inhibition (in addition to hepatic fibrosis) of inflammatory infiltration and hepatocytes apoptosis in the curcumin-compared with the vehicle-treated rats. To further explore potential anti-fibrotic actions of curcumin, we examined whether curcumin would have a beneficial effect on established liver cirrhosis. Such an effect may support a mechanism that includes direct anti-fibrotic or fibrinolytic activity. However, curcumin administration for either 4 or 6 weeks to rats that already received TAA for 12 weeks and were confirmed to be cirrhotic did not improve liver histology, hepatic hydroxyproline levels or the spleen weights, indicating that curcumin had no effect on pre-established liver cirrhosis. These data are consistent with the results in HSC that showed no effect of curcumin on MMP-2 activity. The failure of curcumin to inhibit HSC proliferation and profibrogenic activity in vitro, observed in our study, is not in accordance with a previous study (9). The reason for this discrepancy is not apparent, apart from the use of different HSC cell line.

In summary, curcumin treatment prevented the development of hepatic cirrhosis in rats that were administered the hepatotoxin TAA. As curcumin is safe for consumption by humans, it may have a beneficial role in chronic liver diseases characterized by ongoing hepatic necroinflammation.

References


