

Amin A. Nanji, Kalle Jokelainen, George L. Tipoe, Amir Rahemtulla, Peter Thomas and Andrew J. Dannenberg

Am J Physiol Gastrointest Liver Physiol 284:321-327, 2003. First published Aug 28, 2002;
doi:10.1152/ajpgi.00230.2002

You might find this additional information useful...

This article cites 41 articles, 13 of which you can access free at:

<http://ajpgi.physiology.org/cgi/content/full/284/2/G321#BIBL>

This article has been cited by 3 other HighWire hosted articles:

Curcumin, An Atoxic Antioxidant and Natural NF{ κ }B, Cyclooxygenase-2, Lipoxygenase, and Inducible Nitric Oxide Synthase Inhibitor: A Shield Against Acute and Chronic Diseases

S. Bengmark

JPEN J Parenter Enteral Nutr, January 1, 2006; 30 (1): 45-51.

[\[Abstract\]](#) [\[Full Text\]](#) [\[PDF\]](#)

Curcumin Inhibits Platelet-Derived Growth Factor-Stimulated Vascular Smooth Muscle Cell Function and Injury-Induced Neointima Formation

X. Yang, D. P. Thomas, X. Zhang, B. W. Culver, B. M. Alexander, W. J. Murdoch, M. N.A. Rao, D. A. Tulis, J. Ren and N. Sreejayan

Arterioscler. Thromb. Vasc. Biol., January 1, 2006; 26 (1): 85-90.

[\[Abstract\]](#) [\[Full Text\]](#) [\[PDF\]](#)

Recent Insights into the Role of the Innate Immune System in the Development of Alcoholic Liver Disease

L. E. Nagy

Experimental Biology and Medicine, September 1, 2003; 228 (8): 882-890.

[\[Abstract\]](#) [\[Full Text\]](#) [\[PDF\]](#)

Updated information and services including high-resolution figures, can be found at:

<http://ajpgi.physiology.org/cgi/content/full/284/2/G321>

Additional material and information about *AJP - Gastrointestinal and Liver Physiology* can be found at:

<http://www.the-aps.org/publications/ajpgi>

This information is current as of October 8, 2007 .

Curcumin prevents alcohol-induced liver disease in rats by inhibiting the expression of NF- κ B-dependent genes

AMIN A. NANJI,¹ KALLE JOKELAINEN,² GEORGE L. TIPOE,³ AMIR RAHEMTULLA,⁴ PETER THOMAS,⁵ AND ANDREW J. DANNENBERG⁶

¹Department of Pathology and Laboratory Medicine, University of Pennsylvania Medical Center, Philadelphia, Pennsylvania 19104-4283; ²Research Unit of Alcohol Diseases, Helsinki University Central Hospital, Helsinki, Finland; ³Department of Anatomy, The University of Hong Kong, Hong Kong; ⁴Department of Pathology, Harvard Medical School, Boston 02115; ⁵Department of Surgery, Boston University School of Medicine, Boston, Massachusetts 02118; and ⁶Department of Medicine, Weill Medical College of Cornell University and Anne Fisher Nutrition Center at Strang Cancer Prevention Center, New York, New York 10021

Submitted 14 June 2002; accepted in final form 22 August 2002

Nanji, Amin A., Kalle Jokelainen, George L. Tipoe, Amir Rahemtulla, Peter Thomas, and Andrew J. Dannenberg. Curcumin prevents alcohol-induced liver disease in rats by inhibiting the expression of NF- κ B-dependent genes. *Am J Physiol Gastrointest Liver Physiol* 284: G321–G327, 2003. First published August 28, 2002; 10.1152/ajpgi.00230.2002.—Induction of NF- κ B-mediated gene expression has been implicated in the pathogenesis of alcoholic liver disease (ALD). Curcumin, a phenolic antioxidant, inhibits the activation of NF- κ B. We determined whether treatment with curcumin would prevent experimental ALD and elucidated the underlying mechanism. Four groups of rats (6 rats/group) were treated by intragastric infusion for 4 wk. One group received fish oil plus ethanol (FE); a second group received fish oil plus dextrose (FD). The third and fourth groups received FE or FD supplemented with 75 mg·kg⁻¹·day⁻¹ of curcumin. Liver samples were analyzed for histopathology, lipid peroxidation, NF- κ B binding, TNF- α , IL-12, monocyte chemotactic protein-1, macrophage inflammatory protein-2, cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), and nitrotyrosine. Rats fed FE developed fatty liver, necrosis, and inflammation, which was accompanied by activation of NF- κ B and the induction of cytokines, chemokines, COX-2, iNOS, and nitrotyrosine formation. Treatment with curcumin prevented both the pathological and biochemical changes induced by alcohol. Because endotoxin and the Kupffer cell are implicated in the pathogenesis of ALD, we investigated whether curcumin suppressed the stimulatory effects of endotoxin in isolated Kupffer cells. Curcumin blocked endotoxin-mediated activation of NF- κ B and suppressed the expression of cytokines, chemokines, COX-2, and iNOS in Kupffer cells. Thus curcumin prevents experimental ALD, in part by suppressing induction of NF- κ B-dependent genes.

antioxidants; cyclooxygenase; nitric oxide

THERE IS CONSIDERABLE EVIDENCE that endotoxin and oxidative stress contribute to alcoholic liver disease (ALD)

Address for reprint requests and other correspondence: A. A. Nanji, Dept. of Pathology and Laboratory Medicine, Univ. of Pennsylvania School of Medicine, 7.046 Founders Pavilion, 3400 Spruce St., Philadelphia, PA 19104-4283 (E-mail: amin.nanji@uphs.upenn.edu).

(14, 15, 25, 27). For example, chronic alcohol administration results in increased levels of endotoxin in the portal circulation, thereby activating Kupffer cells to produce toxic mediators that cause liver injury (24, 37). Transcription NF- κ B, a key regulator of genes involved in inflammation, is activated by endotoxin or oxidative stress (2). In unstimulated cells, NF- κ B is sequestered in the cytoplasm by association with the inhibitor κ B (I κ B) family of inhibitors. After stimulation, I κ B undergoes phosphorylation, ubiquitination, and subsequent proteolysis, enabling NF- κ B to translocate to the nucleus, in which it regulates the transcription of target genes (2, 3, 18). Numerous target genes have been implicated in the pathogenesis of ALD, including TNF- α , IL-12, monocyte chemotactic protein-1 (MCP-1), macrophage inflammatory protein-2 (MIP-2), inducible nitric oxide synthase (iNOS), and cyclooxygenase-2 (COX-2) (19, 21, 23, 26, 29). Recently, work was carried out to test the hypothesis that inhibiting the activation of NF- κ B would protect against experimental ALD (39). Inhibition of NF- κ B by delivery of an adenoviral vector encoding the transgene for an I κ B superrepressor prevented alcohol-induced liver injury (39). On the basis of this finding, it is of considerable importance to determine whether pharmacological approaches can be developed to suppress the activation of NF- κ B and thereby prevent ALD.

Phenolic antioxidants are a class of dietary compounds that possess anti-inflammatory properties (13). Curcumin (diferuloylmethane) is a representative phenolic antioxidant found in the dietary spice turmeric (Fig. 1) (6). It is derived from the rhizome of the plant *Curcuma longa* and exhibits antioxidant, anti-inflammatory, and anticancer properties (40). Curcumin has been used as a folk remedy and is currently undergoing clinical testing in humans (4). Importantly, it has been observed to suppress the activation of NF- κ B (30, 33,

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

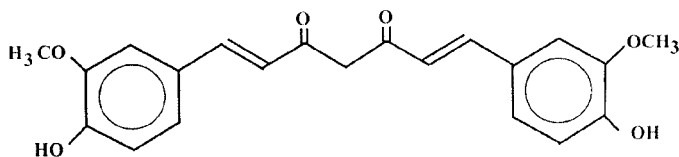


Fig. 1. Structure of curcumin.

36), raising the possibility that it might be useful in preventing ALD.

The principal goal of the present study was to determine whether treatment with curcumin could suppress the expression of NF- κ B-dependent genes and prevent ALD. The intragastric feeding rat model for ALD was used, because it is well established and permits the correlation of histological and biochemical parameters (8, 9, 38). Evidence is presented that curcumin prevented ALD, at least in part, by inhibiting lipid peroxidation, activation of NF- κ B, and expression of pro-inflammatory mediators. Consistent with previous findings (37, 39), the Kupffer cell appears to be centrally involved in this process.

MATERIALS AND METHODS

Animal model and isolation of Kupffer cells. Male Wistar rats (Harlan Sprague Dawley, Indianapolis, IN) weighing between 225 and 250 g were fed a liquid diet by continuous infusion through permanently implanted gastric tubes as previously described (9, 38). Ethanol and diet were administered continuously by a single gastric cannula. This was achieved by joining two tubes, one carrying ethanol from one syringe pump and the other carrying diet from a second pump, so that ethanol and diet could be varied at will. The dose of ethanol was increased slowly, as tolerance developed, to maintain blood alcohol levels in the range of 150–300 mg/dl. The starting dose was $8 \text{ g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$; the final dose was $16 \text{ g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$. Each ethanol-fed rat had 4–6 measurements of blood alcohol; the blood sample was obtained at 10 AM.

Four groups (6 rats/group) of rats were treated for 4 wk before being killed. Rats in the first group were administered fish oil plus ethanol (FE). This diet is known to cause ALD in the rat (25). A second group of rats was fed fish oil plus dextrose (FD). The third and fourth groups received FE or FD supplemented with $75 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ curcumin (Sigma, St. Louis, MO). This dose of curcumin has been used safely in previous studies (6, 41). The curcumin was stored in opaque containers to protect it from light. All animals received humane care in compliance with the *Guide for the Care and Use of Laboratory Animals* [DHEW Publication No. (NIH) 85-23, Revised 1985, Office of Science and Health Reports, DRR/NIH, Bethesda, MD 20205]. Kupffer cells were isolated from livers of male Sprague-Dawley rats using previously established procedures (12).

Histopathological analysis. A small sample of liver was obtained at death and fixed in formalin. Hematoxylin-eosin stain was used for light microscopy. The severity of liver pathology was assessed as steatosis (the percentage of liver cells containing fat): 1+, $\leq 25\%$; 2+, 26–50%; 3+, 51–75%; 4+, $> 75\%$. Necrosis was evaluated as the number of necrotic foci per square millimeter; inflammation was scored as the number of inflammatory cells per square millimeter. At least three different sections were examined per sample of liver.

Measurements of blood alcohol, serum alanine aminotransferase, conjugated dienes, and thiobarbituric acid-reacting

substances. Blood was collected from the tail vein, and ethanol concentration was measured using a kit from Sigma. Serum alanine aminotransferase (ALT) was measured using an automated analyzer (model Hitachi 747; Boehringer-Mannheim, Indianapolis, IN) (8). Conjugated dienes and thiobarbituric acid-reacting substances (TBARS) were measured as previously described (31).

Determination of NF- κ B binding activity and levels of I κ B α in liver tissue and Kupffer cells. The livers were fractionated, and nuclear extracts were used to determine NF- κ B binding activity as described previously (16, 17, 23). Liver cytosols were used to determine amounts of I κ B α . Nuclear fractions were obtained from Kupffer cells as described previously (12) by using an adaptation of the method of Schrieber et al. (35). As in previous studies (23), specificity of NF- κ B binding was confirmed by competition assays and the ability of a specific antibody to supershift protein-DNA complexes. In competition assays, the addition of a 100-fold excess of unlabeled competitor consensus oligonucleotide prevented binding. Supershift experiments confirmed the presence of the p50 subunit in the binding complex. Western blot analysis for I κ B α was conducted using $50 \mu\text{g}$ of cytosolic protein. Samples were electrophoresed on a 10% sodium dodecyl sulfate-polyacrylamide gel. After transfer, the nitrocellulose membrane was incubated with an anti-I κ B α antibody (Santa Cruz Biotechnology, Santa Cruz, CA) at a dilution of 1:500. Membranes were incubated with a secondary antibody (horseradish peroxidase-conjugated goat anti-rabbit IgG). Antibody-reactive bands were visualized by the use of an enhanced chemiluminescence assay using reagents from New England Nuclear-Life Science Products (Wilmington, DE).

Analysis of mRNAs for COX-1, COX-2, iNOS, TNF- α , IL-12, MCP-1, MIP-2, and β -actin by RT-PCR. To examine the expression of COX-1, COX-2, iNOS, TNF- α , IL-12, MCP-1, MIP-2, and β -actin in liver tissue and Kupffer cells, total RNA was isolated according to the guanidium isothiocyanate method (6). Integrity of RNA was assessed by agarose gel electrophoresis and ethidium bromide staining. RT-PCR was performed essentially as described previously (26). The sequences of primer pairs (5' and 3') and PCR conditions have been reported previously (21, 22, 28, 29). To normalize signals from the different RNA samples, we amplified $2 \mu\text{l}$ of the same reverse-transcriptase reaction with β -actin-specific primers. PCR products and molecular weight markers were subjected to electrophoresis on 1% agarose gels and visualized by means of ethidium bromide staining.

Immunohistochemical analysis. Sections were immunostained with antiserum to iNOS and nitrotyrosine using a DAKO Envision kit. Sections were deparaffinized in xylene and rehydrated through graded ethanol concentrations. To block endogenous peroxidase activity, the sections were immersed in 3% hydrogen peroxide for 5 min at room temperature. Sections were preincubated with 10% normal horse serum for 1 h to reduce nonspecific binding of the antiserum. The sections were then incubated overnight at 4°C with antibodies to iNOS (Transduction Laboratories, San Diego, CA) and nitrotyrosine (Upstate Biotechnology, New York, NY), respectively. Antibodies were diluted at 1:100 in 0.05 M Tris·HCl buffer containing 2% bovine serum albumin. Sections were washed three times in PBS, then incubated with peroxidase-labeled polymer conjugated to goat anti-rabbit IgG in Tris·HCl for 30 min at 37°C . Finally, sections were washed and the peroxidase was visualized by immersion in 0.05% diaminobenzidine containing 0.03% hydrogen peroxide in Tris·HCl buffer (pH 7.5) for 3 min. Sections were rinsed in distilled water and counterstained with hematoxy-

Table 1. Pathological changes, ALT activity, and lipid peroxidation in the experimental groups

Experimental Group	Fatty Liver, 0–4	Necrosis, foci/mm ²	Inflammation, cells/mm ²	ALT activity, 5–35 U/l	A ₂₃₂ , conjugated dienes	TBARS, nmol/mg protein
Fish oil-dextrose	0	0	0.04 ± 0.02	14 ± 6	0.34 ± 0.07	0.31 ± 0.09
Fish oil-dextrose + curcumin	0	0	0	15 ± 3	0.29 ± 0.08	0.39 ± 0.06
Fish oil + ethanol	4.0 ± 0.0†	1.1 ± 0.5‡	21.7 ± 8.8‡	78 ± 13‡	1.4 ± 0.29‡	1.70 ± 0.22‡
Fish oil + ethanol + curcumin	2.0 ± 0.8*†	0	0	32 ± 6†	0.58 ± 0.09§	0.69 ± 0.04§

Values are means ± SD; *n* = 6 rats. ALT, alanine aminotransferase; A₂₃₂, conjugated dienes; TBARS, thiobarbituric acid-reacting substances. **P* < 0.05 vs. fish oil-ethanol; †*P* < 0.01 vs. dextrose-fed groups; ‡*P* < 0.01 vs. other groups; §*P* < 0.05 vs. dextrose-fed groups.

lin. Positive staining was indicated by a brown color. Control sections were incubated with normal rabbit IgG.

Statistical analysis. Results are presented as means ± SD unless otherwise indicated. Analysis of variance and multiple comparisons with the Student-Newman-Kuels method were used to determine statistical significance, which was set at *P* < 0.05.

RESULTS

In each of the four groups, the rats increased their weight at a constant rate. Moreover, there was no difference in weight gain among the groups. There was also no difference in levels of blood alcohol in the two alcohol-fed groups (FE, 232 ± 37 mg/dl; FE-curcumin, 214 ± 31 mg/dl).

Curcumin inhibits alcohol-induced liver injury and lipid peroxidation. Feeding rats the FE diet for 4 wk caused fatty liver, necrosis, and inflammation (Table 1) (Fig. 2). These histological changes were associated with elevated levels of ALT and lipid peroxidation (conjugated dienes, TBARS) (Table 1). Importantly, treatment with curcumin prevented both

alcohol-induced necrosis and inflammation. The degree of fatty liver also decreased in curcumin-treated rats. Consistent with the improved histology, treatment with curcumin was associated with a corresponding reduction in levels of ALT and lipid peroxidation. Neither histological nor biochemical evidence of liver injury was detected in rats that received FD or FD-curcumin diets.

Effect of curcumin on activation of NF-κB in liver. Electromobility shift assays were carried out to determine NF-κB binding activity in the livers of rats in the different groups. As shown in Fig. 3, the FE diet led to increased NF-κB binding activity compared with the FD diet. Interestingly, curcumin prevented the activation of NF-κB in rats fed the FE diet consistent with its ability to inhibit lipid peroxidation and liver injury. To determine whether activation of NF-κB was a consequence of degradation of IκBα, we determined levels of IκBα in the cytosolic fraction of the liver. Very low levels of IκBα were detected in the FE group (Fig. 3). By contrast, higher

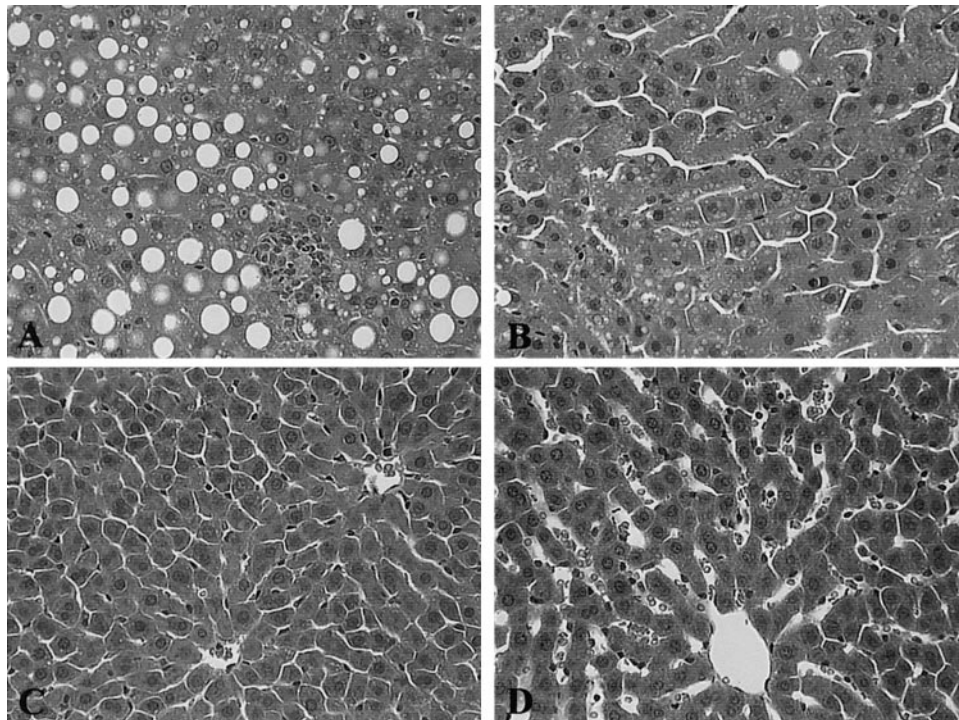


Fig. 2. A: liver from a rat fed fish oil and ethanol for 4 wk shows the presence of fatty liver, necrosis, and inflammation (magnification, ×400). B: liver from a rat fed fish oil ethanol and treated with curcumin. Note the absence of necrosis and inflammation and the reduction in the degree of fatty liver. C and D: livers from rats fed fish oil and dextrose (C) and fish oil-dextrose and curcumin (D) showing normal liver architecture.

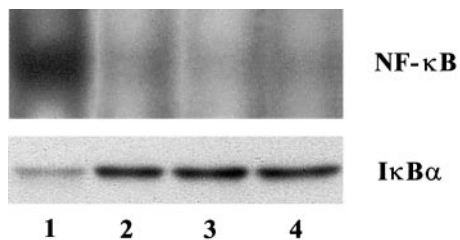


Fig. 3. Curcumin inhibits the activation of NF- κ B in alcoholic liver disease (ALD). Representative electrophoretic mobility shift assay for NF- κ B binding and Western blot analysis for inhibitor κ B α (I κ B α). Increased NF- κ B binding activity was observed in rats fed fish oil and ethanol (FE) (lane 1) compared with rats fed the FE diet supplemented with curcumin (lane 2). Similarly, NF- κ B binding activity was low or undetectable in rats fed fish oil and dextrose (FD) (lane 3) or FD supplemented with curcumin (lane 4). Levels of I κ B α in cytosol were decreased in the FE group compared with other groups.

levels of I κ B α were detected in the other groups including the FE-curcumin group.

Effect of curcumin on TNF- α , IL-12, MCP-1, MIP-2, COX-2, and iNOS mRNAs in liver. Various NF- κ B-responsive genes including TNF- α , IL-12, MCP-1, MIP-2, COX-2 and iNOS are overexpressed in experimental ALD (21, 22, 23). Because the levels of these mRNAs are generally too low to detect by Northern blot or ribonuclease protection assays, RT-PCR was carried out. As predicted from previous studies (21, 22, 23), we detected markedly elevated levels of these proinflammatory mediators in the livers of rats fed FE vs. FD diet (Fig. 4). Remarkably, treatment with curcumin resulted in a normalization of levels of each of the above proinflammatory mediators. Nitric oxide, a free radical product of iNOS, can potentially damage the liver. It is noteworthy, therefore, that there was a marked reduction in nitrotyrosine immunoreactivity in the livers of rats treated with FE-curcumin vs. FE (Fig. 5). Levels of COX-1 mRNA, the constitutive isoform of COX, were similar in all groups (Fig. 4).

Curcumin inhibits endotoxin (LPS)-mediated activation of NF- κ B-responsive genes in Kupffer cells. Endotoxin-mediated activation of gene expression in Kupffer cells has been linked to the pathogenesis of ALD (37, 39). Hence, it was of interest to determine whether the effects observed in whole liver could be reproduced in isolated Kupffer cells. Under basal conditions, we did not detect significant NF- κ B binding activity or iNOS, COX-2, MIP-2, MCP-1, IL-12, and TNF- α message (data not shown). As shown in Fig. 6, treatment with endotoxin stimulated NF- κ B binding and induced the expression of each of the above proinflammatory mediators. This inductive effect of endotoxin was blocked by curcumin, consistent with our findings in whole liver (Fig. 4).

DISCUSSION

Treatment of alcohol-induced liver disease remains limited to supportive measures (10, 20). Undoubtedly, the development of effective therapy to prevent or treat ALD will depend on elucidating the underlying mech-

anisms that contribute to liver injury. Several lines of evidence suggest that the induction of NF- κ B-dependent gene expression in Kupffer cells contributes to alcohol-induced liver injury (15, 16, 27, 39). In support of this hypothesis, gene therapy has been used to inhibit the activation of NF- κ B and thereby prevent experimental ALD (39). Given the current limitations of this approach (42), it would be highly desirable if a pharmacological strategy could be developed to suppress the activation of NF- κ B and prevent ALD.

In the present study, we found that alcohol-induced liver injury was associated with increased amounts of lipid peroxidation and the induction of multiple NF- κ B-dependent genes including TNF- α , IL-12, MCP-1, MIP-2, COX-2, and iNOS. Each of these genes has been implicated in the pathogenesis of liver injury (7, 22, 23, 26, 27, 39). In addition to preventing alcohol-induced necroinflammatory changes, treatment with curcumin prevented lipid peroxidation and the expression of the above NF- κ B-dependent genes. Although curcumin is known to inhibit the activation of NF- κ B and suppress inflammation (30, 33, 36), this is the first time it has been shown to prevent ALD. Whether curcumin can also be used to treat established ALD is uncertain and requires further investigation. Because curcumin can be given safely to humans (32, 34), the results of this study have potentially important therapeutic implications for individuals at risk for ALD. Clinical trials will be necessary to evaluate this question.

Another important issue concerns the locus of action of curcumin. Although NF- κ B appears to be a key mediator of the inflammatory response in Kupffer cells, it functions as a survival factor in hepatocytes under

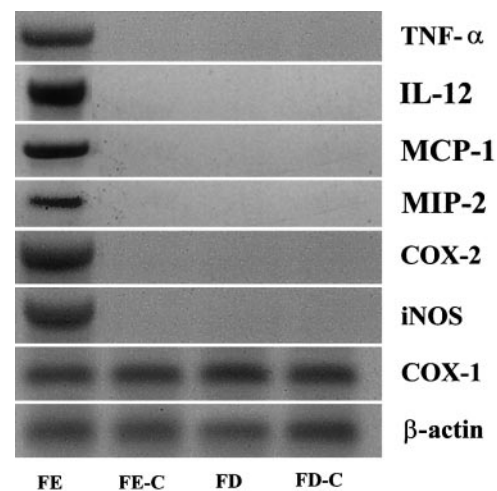


Fig. 4. RT-PCR analysis of monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein-2 (MIP-2), cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), COX-1, and β -actin was carried out in liver samples from the different groups. One microgram of RNA was subjected to reverse transcription as detailed in MATERIALS AND METHODS. PCR products were subjected to electrophoresis on 1% agarose gels and visualized by ethidium bromide staining. mRNAs for cytokines, chemokines, COX-2, and iNOS were detected only in the FE group and not in the other treatment groups. COX-1 was detected in all groups. FE-C, FE supplemented with curcumin; FD-C, FD supplemented with curcumin.

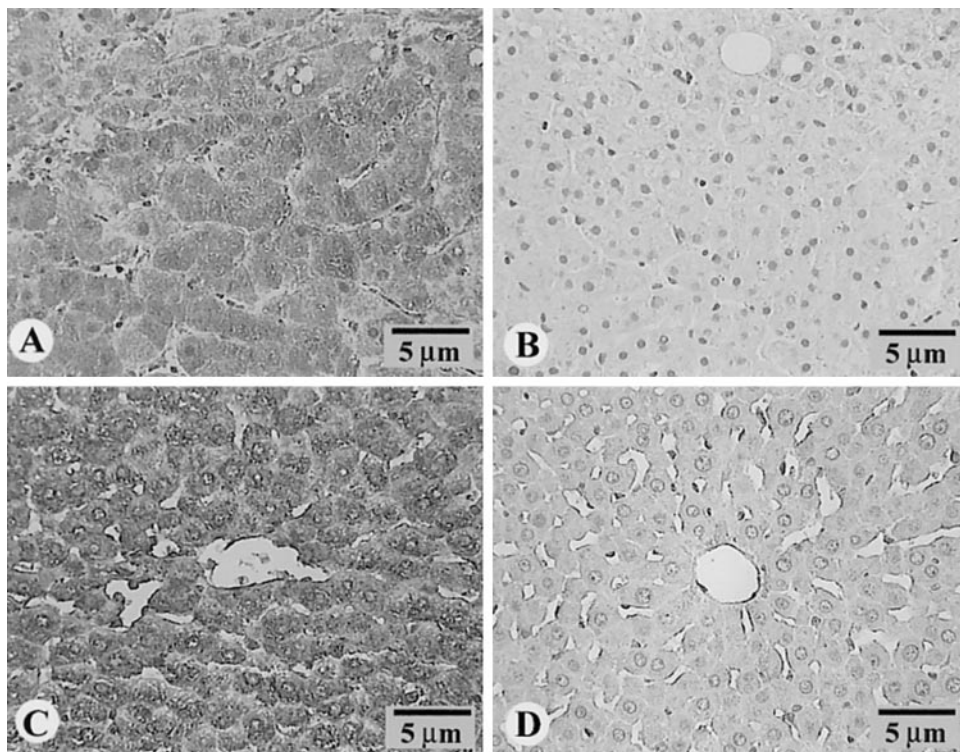


Fig. 5. Immunostaining for iNOS and nitrotyrosine in rats treated with FE compared with FE-C. *A*: strong staining for iNOS was present in the hepatocytes in the FE group. *B*: intensity of staining was decreased in the hepatocytes of FE-C rats. *C* and *D*: degree of staining for nitrotyrosine paralleled the increased staining for iNOS in FE rats (*C*) and was decreased by treatment with curcumin (*D*).

certain physiological conditions, e.g., liver regeneration (42). Consequently, suppression of NF-κB activity can have beneficial or deleterious effects depending on the condition being studied (11). Several investigators have presented evidence that endotoxin-activated Kupffer cells produce toxic mediators, such as proin-

flammatory cytokines and reactive oxygen species, leading to ALD (23, 27, 37). Hence, we investigated whether curcumin blocked endotoxin-mediated induction of NF-κB-dependent gene expression in isolated Kupffer cells. Curcumin caused dose-dependent suppression of endotoxin-mediated induction of NF-κB binding activity (Fig. 6). It also blocked the expression of the same panel of genes in endotoxin-treated Kupffer cells observed in the livers of alcohol-treated

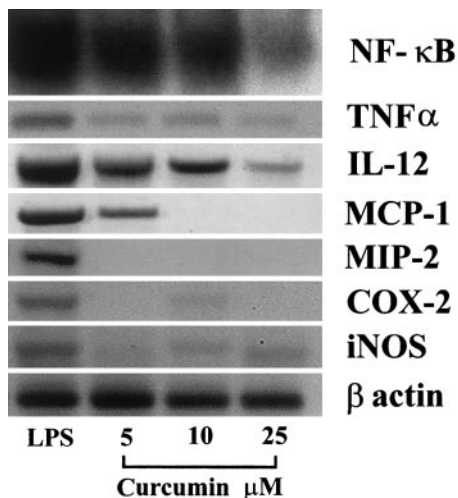


Fig. 6. Curcumin inhibits NF-κB binding activity and the expression of TNF-α, IL-12, MCP-1, MIP-2, COX-2, and iNOS in endotoxin-treated Kupffer cells. Isolated Kupffer cells were treated with vehicle or the indicated concentrations of curcumin for 1 h before treatment with LPS (100 ng/ml). At 15 min after adding LPS, the Kupffer cells were harvested and washed three times with PBS. Electrophoretic mobility shift assays were performed to assess NF-κB binding activity. Levels of mRNA for cytokines, chemokines, COX-2, and iNOS were assessed by RT-PCR. Treatment with curcumin inhibited NF-κB binding activity and the expression of TNF-α, IL-12, MCP-1, MIP-2, COX-2, and iNOS in endotoxin treated cells.

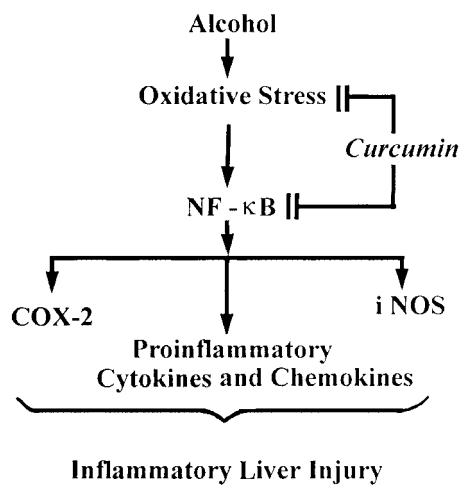


Fig. 7. Schematic illustrating proposed mechanism by which curcumin prevents ALD. Treatment with alcohol causes increased levels of endotoxin in the portal circulation and oxidative stress. This results, in turn, in the activation of multiple NF-κB-responsive genes including proinflammatory cytokines, chemokines, COX-2, and iNOS. Curcumin, a phenolic antioxidant, inhibits lipid peroxidation and the activation of the above NF-κB-responsive genes, thereby protecting against ALD.

rats (Fig. 4). Interestingly, lower concentrations of curcumin were required to maximally suppress levels of iNOS, COX-2, and MIP-2 vs. IL-12 and MCP-1 in endotoxin-treated Kupffer cells. Currently, we do not have an explanation for this difference in response to curcumin. Our data do not permit us to exclude the possibility that curcumin had direct effects on other cell types in the liver, e.g., hepatocytes, in addition to Kupffer cells. In fact, we found that treatment with curcumin led to a reduction in levels of iNOS and nitrotyrosine in hepatocytes. We also cannot exclude the possibility that oxidant stress is increased secondary to activation of NF- κ B (39, 42). Nonetheless, the benefits of suppressing the proinflammatory effects of NF- κ B activation in the Kupffer cell appear to outweigh any potentially detrimental effects involving other cell types.

Reduction in the severity of fatty liver in the curcumin-treated rats should also be noted. Studies in ethanol-fed rats have shown that inhibition of TNF- α leads to a decrease in the amount of fat storage in the liver (37). Other mechanisms, such as induction of enhanced fatty acid catabolism, occur in the livers of rats after curcumin treatment (1). A decrease in hepatic fat accumulation in ethanol-fed rats is also observed in response to antioxidant therapy (14). Thus multiple mechanisms are probably involved in the reduction of the degree of fat accumulation observed in curcumin-treated rats in the present study.

The pathogenesis of ALD is complex. In the present study, we showed that curcumin, a dietary phenolic antioxidant, was highly effective in preventing experimental ALD. In addition to preventing alcohol-induced liver injury, it blocked lipid peroxidation, the activation of NF- κ B, and the expression of proinflammatory cytokines and chemokines, iNOS and COX-2 (Fig. 7). Developing compounds that target specific molecules, such as COX-2, is useful for treating certain inflammatory conditions, such as arthritis. By contrast, this study suggests that agents that prevent the activation of a transcription factor, i.e., NF- κ B, will suppress expression of a series of proinflammatory molecules and thereby prevent ALD. Ultimately, clinical trials will be needed to determine whether agents, such as curcumin, that inhibit the activation of NF- κ B will be effective in preventing and possibly treating alcohol-induced liver injury in humans.

We thank Diana Peters, Timothy Cloutier, and Lili Miao for technical assistance. Catherine Li provided editorial help.

This study was supported by grants from the Research Grants Council of Hong Kong and National Institutes of Health. Dr. Kalle Jokelainen was supported by grants from the Academy of Finland, Finnish Cultural Foundation, and Yrjö Jahnsson Foundation.

REFERENCES

- Asai A and Miyazawa T. Dietary curcuminoids prevent high fat diet-induced lipid accumulation in rat liver and epididymal adipose tissue. *J Nutr* 131: 2932–2935, 2001.
- Baeuerle PA and Baltimore D. NF- κ B: ten years after. *Cell* 87: 13–20, 1996.
- Barnes PJ and Karin M. Nuclear factor κ B—a pivotal transcription factor in chronic inflammatory diseases. *N Engl J Med* 336: 1066–1071, 1997.
- Bravo L. Polyphenols: chemistry, dietary sources, metabolism and nutritional significance. *Nutr Rev* 56: 317–333, 1998.
- Chomeczynski P and Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* 162: 156–159, 1987.
- Commandeur JNM and Vermeulen NPE. Cytotoxicity and cytoprotective activities of natural compounds: the case of curcumin. *Xenobiotica* 26: 667–680, 1996.
- Dinchuk JE, Car BD, Focht RJ, Johnson JJ, Jaffee BD, Covington MB, Contel NR, Eng VM, Collins RJ, Czerniac P, and Trzaskos JM. Renal abnormalities and an altered inflammatory response in mice lacking cyclooxygenase-2. *Nature* 378: 406–409, 1995.
- French SW, Miyamoto K, and Tsukamoto H. Ethanol-induced hepatic fibrosis in the rat: role of the amount of dietary fat. *Alcohol Clin Exp Res* 10, Suppl 6: 13S–19S, 1986.
- French SW, Wong K, Jui L, Albano E, Hagbjork AL, and Ingelman-Sundberg M. Effect of ethanol on cytochrome P450 2E1 (CYP 2E1), lipid peroxidation and serum protein adduct formation in relation to liver pathology pathogenesis. *Exp Mol Pathol* 58: 61–75, 1993.
- Fulton S and McCullough AJ. Treatment of alcoholic hepatitis. *Clin Liver Dis* 2: 779–819, 1998.
- Iimuro Y, Nishiura T, Hellerbrand C, Behrens KE, Schoonhoven R, Grisham JW, and Brenner DA. NF κ B prevents apoptosis and liver dysfunction during liver regeneration. *J Clin Invest* 101: 802–811, 1998.
- Jokelainen K, Thomas P, Lindros K, and Nanji AA. Acetaldehyde inhibits NF- κ B activation through I κ B- α preservation in rat Kupffer cells. *Biochem Biophys Res Commun* 253: 834–836, 1998.
- Kagan VE and Tyurina YY. Recycling and redox cycling of phenolic antioxidants. *Ann NY Acad Sci* 854: 425–434, 1998.
- Kaplowitz N and Tsukamoto H. Oxidative stress and liver disease. *Prog Liver Dis* 14: 131–159, 1996.
- Lieber CS. Alcohol and the liver: 1994 update. *Gastroenterology* 106: 1085–1105, 1994.
- Lin M, Rippe RA, Niemela O, Brittenham G, and Tsukamoto H. Role of iron in NF- κ B activation and cytokine gene expression by rat hepatic macrophages. *Am J Physiol Gastrointest Liver Physiol* 272: G1355–G1364, 1997.
- Liu SL, Esposti SD, Yao L, Diehl AM, and Zern MA. Vitamin E therapy for acute CCl₄-induced hepatic injury is associated with inhibition of nuclear factor kappa B binding. *Hepatology* 22: 1474–1481, 1995.
- May MJ and Ghosh S. Rel/NF- κ B and I κ B proteins—an overview. *Semin Cancer Biol* 8: 63–73, 1997.
- McClain CJ, Hill D, Schmidt J, and Diehl AM. Cytokines and alcoholic liver disease. *Semin Liver Dis* 13: 170–182, 1993.
- Mullen KD and Dasarthy S. Potential new therapies for alcoholic liver disease. *Clin Liver Dis* 2: 851–881, 1998.
- Nanji AA, Greenberg SS, Tahan SR, Fogt F, Loscalzo J, Sadrzadeh SMH, Xie J, and Stamler JS. Nitric oxide production in experimental alcoholic liver disease in the rat: role in protection from injury. *Gastroenterology* 109: 899–907, 1995.
- Nanji AA, Jokelainen K, Fotouhinia M, Rahemtulla A, Thomas P, Tipoe GL, Su GL, and Dannenberg AJ. Increased severity of alcoholic liver injury in female rats: role of oxidative stress, endotoxin, and chemokines. *Am J Physiol Gastrointest Liver Physiol* 281: G1348–G1356, 2001.
- Nanji AA, Jokelainen K, Rahemtulla A, Miao L, Fogt F, Matsumoto H, Tahan SR, and Su GL. Activation of nuclear factor kappa B and cytokine imbalance in experimental alcoholic liver disease in the rat. *Hepatology* 30: 934–943, 1999.
- Nanji AA, Khettry U, Sadrzadeh SMH, and Yamanaka T. Severity of liver injury in experimental alcoholic liver disease. Correlation with plasma endotoxin, prostaglandin E₂, leukotriene B₄, and thromboxane B₂. *Am J Pathol* 142: 367–373, 1993.
- Nanji AA, Khwaja S, Tahan SR, and Sadrzadeh SMH. Plasma levels of a novel noncyclooxygenase-derived prostanoid (8-isoprostane) correlate with severity of liver injury in experi-

- mental alcoholic liver disease. *J Pharmacol Exp Ther* 269: 1280–1285, 1994.
26. **Nanji AA, Miao L, Thomas P, Rahemtulla A, Khwaja S, Zhao S, Peters D, Tahan SR, and Dannenberg AJ.** Enhanced cyclooxygenase-2 gene expression in alcoholic liver disease in the rat. *Gastroenterology* 112: 943–951, 1997.
 27. **Nanji AA and Zakim D.** Alcoholic liver disease. In: *Hepatology: A Textbook of Liver Disease*, edited by Zakim D and Boyer TD (3rd ed.). Philadelphia, PA: Saunders, 1996, p. 891–961.
 28. **Nanji AA, Zakim D, Rahemtulla A, Daly T, Miao L, Zhao S, Khwaja S, Tahan SR, and Dannenberg AJ.** Dietary saturated fatty acids down-regulate cyclooxygenase-2 and tumor necrosis factor- α and reverse fibrosis in alcohol-induced liver disease in the rat. *Hepatology* 26: 1538–1545, 1997.
 29. **Nanji AA, Zhao S, Sadrzadeh SMH, and Waxman DJ.** Use of reverse transcriptase-polymerase chain reaction to evaluate in vivo cytokine gene expression in rats fed ethanol for long periods. *Hepatology* 19: 1483–1487, 1994.
 30. **Pan MH, Lin-Shiau SY, and Lin JK.** Comparative studies on the suppression of nitric oxide synthase by curcumin and its hydrogenated metabolites through down-regulation of IkappaB kinase and NFkappaB activation in macrophages. *Biochem Pharmacol* 60: 1665–1676, 2000.
 31. **Polavarapu R, Spitz DR, Sim JE, Follansbee MH, Oberly LW, Rahemtulla A, and Nanji AA.** Increased lipid peroxidation and impaired antioxidant enzyme function is associated with pathological liver injury in experimental alcoholic liver disease in rats fed diets high in corn oil and fish oil. *Hepatology* 27: 1317–1323, 1998.
 32. **Plummer SM, Hill KA, Festing MFW, Steward WP, Gescher AJ, and Sharma RA.** Clinical development of leukocyte cyclooxygenase-2 activity as a systemic biomarker for cancer chemopreventive agents. *Cancer Epidemiol Biomarkers Prev* 10: 1295–1299, 2001.
 33. **Plummer SM, Holloway KA, Manson MM, Munks R JL, Kaptein A, Farrow S, and Howells L.** Inhibition of cyclooxygenase-2 expression in colon cells by the chemopreventive agent curcumin involves inhibition of NF- κ B activation via NIK/IKK signaling complex. *Oncogene* 18: 6013–6020, 1999.
 34. **Ramirez-Bosca A, Soler A, Gutierrez MA, Alvarez JL, and Almagro EQ.** Antioxidant curcumin extracts decrease the blood lipid peroxide levels in human subjects. *Age* 18: 167–169, 1995.
 35. **Schreiber E, Matthias P, Muller MM, and Schaffner W.** Rapid detection of octamer binding proteins with “mini-extracts” prepared from a small number of cells. *Nucleic Acids Res* 17: 6419, 1989.
 36. **Singh S and Aggarwal BB.** Activation of transcription factor-NF- κ B is suppressed by curcumin (diferuloylmethane). *J Biol Chem* 270: 24995–25000, 1995.
 37. **Thurman RG.** Mechanisms of Hepatic Toxicity. II. Alcoholic liver injury involves activation of Kupffer cells by endotoxin. *Am J Physiol Gastrointest Liver Physiol* 275: G605–G611, 1998.
 38. **Tsukamoto H, Gaal K, and French SW.** Insight into the pathogenesis of alcoholic liver necrosis and fibrosis. *Hepatology* 12: 599–608, 1990.
 39. **Uesegi T, Froh M, Arteil GE, Bradford BU, Gabele E, Wheeler MD, and Thurman RG.** Delivery of IkB superrepressor gene with adenovirus reduces early alcohol-induced liver injury in rats. *Hepatology* 34: 1149–1157, 2001.
 40. **Vinson JA.** Flavonoids in food as in vitro and in vivo antioxidants. *Adv Exp Med Biol* 439: 151–164, 1998.
 41. **Wahlstrom B and Blennow G.** A study on the fate of curcumin in the rat. *Acta Pharmacol Toxicol (Copenh)* 43: 86–92, 1978.
 42. **Yamamoto Y and Gaynor RB.** Therapeutic potential of inhibition of NF- κ B pathway in the treatment of inflammation and cancer. *J Clin Invest* 107: 135–142, 2001.