Attenuation of CCl₄-induced hepatic fibrosis by GdCl₃ treatment or dietary glycine

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The amino acid glycine has been used in several recent studies to prevent various forms of liver injury. For example, glycine minimized endothelial cell death due to reperfusion injury (49), improved graft function, and increased survival after orthotopic liver transplantation (3). In a rat model of endotoxin shock, dietary supplementation with glycine blunted liver and lung injury and improved survival (19). Furthermore, dietary glycine prevented necrosis and inflammation that developed early during chronic intragastric ethanol administration (17). Feeding a glycine-rich diet to rats after 4 wk of alcohol exposure also significantly enhanced the rate of recovery from early alcohol-induced liver injury (47). Therefore, dietary supplementation with glycine may be an effective therapy against liver damage, including injury caused by chronic exposure to alcohol. The mechanism of protection against injury afforded by glycine most likely involves the inactivation of Kupffer cells (19). Recent work (19) demonstrated that glycine prevents increases in intracellular calcium and cytokine production in isolated Kupffer cells exposed to endotoxin. Inhibition of calcium signaling is most likely due to hyperpolarization of the cell membrane via activation of a glycine-gated chloride channel (20). However, the effects of glycine on fibrosis have not been investigated.

The intragastric model of long-term ethanol feeding developed by Tsukamoto and French produces liver injury that closely resembles the pathology observed in human alcoholics (43); however, in rats, long periods of time and supplementation with carbonyl iron are nec-

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necessary to produce consistent cirrhotic lesions with alcohol (41). In contrast, fibrosis models based on the use of chemicals such as CCl₄ provide an efficient way of producing cirrhosis over short periods of time. For example, Proctor and Chatamra (32) developed a model using CCl₄ and phenobarbital that produces standardized micronodular cirrhosis in 75% of rats within 8–10 wk. In the present study, this model of CCl₄-induced cirrhosis was used, along with simultaneous treatment with GdCl₃ or glycine, to determine whether Kupffer cells are involved in the pathogenesis of liver cirrhosis in vivo. The data presented here are consistent with the hypothesis that destruction or inactivation of Kupffer cells blunts the development of fibrosis in the liver in vivo. Preliminary accounts of this work have appeared elsewhere (45).

MATERIALS AND METHODS

Animals. The protocol of animal treatment used in this study was approved by the institutional animal care and use committee. Outbred male Wistar/Han rats (225–250 g; Charles River, Raleigh, NC) were maintained on a 12:12-h light/dark cycle and given unlimited access to standard laboratory chow and water. Beginning 2 wk before treatment with CCl₄, the rats were given water containing 35 mg/dl of phenobarbital. To investigate whether Kupffer cells are involved in fibrosis in vivo, the selective Kupffer cell toxicant GdCl₃ (10 mg/kg) was dissolved in acidic saline and administered to rats twice each week via the tail vein beginning 1 wk before CCl₄ treatment (1). Alternatively, the effects of Kupffer cell inactivation were studied by feeding standard laboratory chow supplemented with 5% casein (nitrogen control) or 5% glycine beginning 2 wk before treatment with CCl₄. Data collected from rats treated with olive oil vehicle (control) or 5% glycine beginning 2 wk before treatment with CCl₄ were not different; therefore, data from the control groups were combined. Regression of fibrosis. Rats were treated with CCl₄ as described in CCl₄ treatment and allowed unlimited access to standard laboratory chow and drinking water containing 35 mg/dl phenobarbital. After 9 wk, CCl₄ and phenobarbital treatment were discontinued. Fibrosis was scored as described in Histology with wedge biopsies of liver collected 3 days after the last injection of CCl₄ to establish maximal fibrosis values. Subsequently, rats were divided into two dietary treatment groups and fed chow supplemented with 5% casein or 5% glycine. Liver biopsies were collected at 4-wk intervals for 12 wk; rats were killed 20 wk after the last injection of CCl₄. Biopsied tissues were stained with trichrome to evaluate fibrosis as described above.

Histology. Liver samples collected after 9 wk of CCl₄ treatment were stained with trichrome, and fibrosis was scored according to the following scoring system modified from Nanji et al. (28): 1 = thickened perivenular collagen and a few thin collagen septa; 2 = thin septa with incomplete bridging between portal regions; 3 = thin septa and extensive bridging; 4 = thickened septae with complete bridging of portal regions and a nodular appearance. Collagen content was measured with a Universal Imaging (Chester, PA) analysis system as described previously (2). Briefly, trichrome-stained liver sections were analyzed with an Axioskope 50 microscope (Carl Zeiss, Thornwood, NY). An intensely labeled point was chosen to set the range of color detection for the blue trichrome stain. Collagen accumulation was calculated as the percentage of the total field at x40 magnification that was stained blue. Fibrosis scores and collagen content in CCl₄-treated rats fed chow or casein were not different; therefore, data from the control groups were combined.

Measurement of a(I) collagen mRNA. Total RNA was harvested from liver tissue as described previously (37). Radiolabeled riboprobes for the RNase protection assay were derived from the 375-nucleotide PsI-Ala fragment of rat a(I) collagen cDNA (39). The riboprobe for the rat GAPDH gene was generated from the plasmid pTRI-GAPDH-Rat (Ambion, Austin, TX), which was linearized with HindIII. Radiolabeled probes were mixed with 25 µg of total liver RNA, and the dried pellets were suspended in 30 µl of hybridization buffer (100 mM PIPES, pH 6.7, 400 mM NaCl, 2 mM EDTA, and 80% formamide). Samples were heated at 85°C for 10 min and then incubated at 45°C overnight. The hybridization reaction was then performed at 37°C for 1 h in RNase buffer (300 mM NaCl, 10 mM Tris-HCl, pH 7.6, 40 µg/ml RNase A, and 2 mg/ml RNase T1). Subsequently, 20% SDS and protease K (10 mg/ml) were added, and the reaction mixture was incubated at 37°C for an additional 15 min. The reaction mixture was extracted with phenol and precipitated with the addition of yeast tRNA and 100% ethanol. The samples were suspended in formamide dye (95% formamide, 20 mM EDTA, 0.05% bromophenol blue, and 0.05% xylene cyanol FF) and loaded onto a standard 6% sequencing gel. After electrophoresis, bands were visualized by autoradiography and quantitated with a PhosphorImager (Molecular Dynamics, Sunnyvale, CA).

TGF-β measurement. Frozen liver samples (500 mg) were homogenized on ice in PBS containing 1 mg/ml of aprotinin (Sigma) and 348 µg/ml of phenylmethylsulfonyl fluoride (Boehringer Mannheim). Samples were then centrifuged at 3,900 g for 10 min. For determination of total TGF-β1, additional supernatant samples were treated with 1 N HCl to convert latent TGF-β1 to the active form. Active and total TGF-β1 were measured by bioassay as described previously (14). Briefly, fibroblasts stably transfected with a TGF-β1

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<th>Δ Weight, g</th>
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<td>Increase 6–10</td>
<td>Last dose + 32 mg</td>
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<td>Increase &gt;10</td>
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<td>Decrease 1–5</td>
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<td>Decrease 6–10</td>
<td>50% of last dose</td>
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<td>Decrease &gt;10</td>
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The initial dose of CCl₄ given was 412 mg/kg. Thereafter, the dose of CCl₄ administered was varied according to changes (Δ) in body weight during the previous week (32).
response element that stimulates luciferase activity were incubated in the presence of the supernatant isolated from liver homogenates. After 3 h, the supernatant was aspirated and luciferase lysis buffer and luciferase substrate buffer were added. Chemiluminescence was measured with a Hamamatsu camera and compared with signals obtained with TGF-β1 standards.

**Immunohistochemistry.** Three days after the last injection of CCl$_4$, rats were given a final dose of GdCl$_3$ or saline and sections of liver were fixed in 10% paraformaldehyde. The effect of GdCl$_3$ on Kupffer cells was investigated by staining 4-μm-thick liver slices with anti-ED1 antibody (BioSource International, Camarillo, CA). Briefly, tissue sections were deparaffinized, and endogenous peroxidase activity was blocked by incubation of tissue in 3% H$_2$O$_2$ for 10 min. Primary anti-ED1 antibody was applied at room temperature for 20 min; rabbit IgG was used as a negative control. ED1-positive cells were detected with a standard immunoenzymatic staining technique (DAKO EnVision/HRP; DAKO, Carpinteria, CA), and sections were counterstained with hematoxylin. Additional liver slices were used for immunohistochemical detection of α-smooth muscle actin (α-SMA), a marker of stellate cell activation, with mouse α-SMA antibody (DAKO). The extent of α-SMA staining was scored with the following system: 1 = slight staining confined to portal areas; 2 = moderate staining in portal areas and light staining along collagen septa; 3 = more intense staining with incomplete bridging between portal areas; and 4 = extensive staining with complete bridging between portal areas.

**Endotoxin measurement.** Heparinized blood samples were drawn from the tail vein at 2-wk intervals during CCl$_4$ treatment and from the portal vein at 9 wk, just before death. Samples were centrifuged at 150 g for 10 min, and the plasma was stored at −80°C. Plasma samples were diluted 1:10 and heated to 75°C for 10 min to denature proteins that interfere with the assay (10). Endotoxin was measured with a kinetic test that used a chromogenic substrate based on the Limulus amebocyte lysate assay (BioWhittaker). Pyrogen-free water and pooled normal rat plasma were used as controls. The concentration of endotoxin in each sample was calculated from a standard curve prepared for each assay in plasma from untreated rats (33). The tubes used for sample collection, storage, and assay preparation were of borosilicate glass that was heated to 200°C for 24 h to destroy endotoxin. Strict nonpyrogenic technique was used for sample collection and for the assay procedure to prevent contamination by exogenous endotoxin (33).

**Measurement of p-nitrophenol metabolism.** To assess cytochrome P-450 (CYP2E1) activity, microsomes were prepared by differential centrifugation from frozen liver samples collected after 9 wk of CCl$_4$ exposure as described previously (24). The rate of hydroxylation of the CYP2E1-specific substrate p-nitrophenol was assessed in isolated microsomes as described elsewhere (23).

**Statistical analysis.** Data were analyzed by one-way ANOVA or two-way ANOVA for repeated measures where appropriate, with $P < 0.05$ selected before the study as the level of significance. All data are presented as means ± SE of at least 4 observations/group.

**RESULTS**

**Destruction of Kupffer cells with GdCl$_3$.** After 9 wk of treatment with CCl$_4$ or CCl$_4$ plus GdCl$_3$, liver sections were stained with anti-ED1 antibody and analyzed by light microscopy. There were $\sim 31 \pm 7$ ED1-positive cells/50 mm$^2$ area in livers from rats treated with CCl$_4$.

In rats treated with GdCl$_3$, the number of ED1-positive cells was reduced by $\sim 75\%$. These data are consistent with previous findings by Hardonk et al. (13) and Koop et al. (24), who showed that injection of GdCl$_3$ destroyed $\sim 80\%$ of all Kupffer cells.

**Effect of GdCl$_3$ and glycine on liver pathology.** In this study, the body weight of each rat was monitored weekly. During 9 wk of treatment, comparable body weight gains were observed in rats receiving CCl$_4$, CCl$_4$ plus GdCl$_3$, and CCl$_4$ plus glycine (Fig. 1). Hence, the total amount of CCl$_4$ administered to each group was similar because of the experimental design. Representative photomicrographs of changes in the liver after CCl$_4$ exposure are shown in Fig. 2. In vehicle-treated controls, only minimal collagen staining was present; no fibrosis was detected in this group (Fig. 2A). In CCl$_4$-treated rats, there was extensive collagen deposition, with septa bridging portal regions as expected (Fig. 2B); the fibrosis score in this group was 2.9 ± 0.1. In contrast, the appearance of bridging collagen fibers was prevented almost completely in rats treated with CCl$_4$ plus GdCl$_3$ (Fig. 2C). Inactivation of Kupffer cells by dietary supplementation with glycine also improved the histological appearance of the liver (Fig. 2D) and significantly reduced the development of fibrosis (Fig. 3).

Collagen is the most abundant extracellular matrix protein produced during fibrogenesis (34). Here, he-

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**Fig. 1.** Effect of CCl$_4$ on average weekly body weight. Male Wistar rats were treated with phenobarbital (35 mg/dl) and CCl$_4$ as described in MATERIALS AND METHODS. In addition, rats were treated with GdCl$_3$ (10 mg/kg iv; A) or a 5% glycine diet (B) starting 2 wk before treatment with CCl$_4$. Untreated control animals displayed comparable changes in body weight (data not shown). Values are means ± SE. No significant differences were observed among the treatment groups by two-way ANOVA for repeated measures.
patic collagen content was estimated in liver sections by using the scoring method described in MATERIALS AND METHODS (Fig. 3). After 9 wk of CCl₄ exposure, the hepatic collagen score increased to 3.0 ± 0.1 in the microscope field (Fig. 3). This increase due to CCl₄ was blunted significantly by GdCl₃ (1.1 ± 0.3) as well as by glycine (1.4 ± 0.3).

**Effects of glycine on recovery from fibrosis.** It is possible that the protective effects of glycine may be due either to enhanced collagen breakdown or to diminished collagen synthesis. To determine the effect of glycine on collagen degradation, the rate of recovery from CCl₄-induced fibrosis was investigated. After CCl₄ was administered to chow-fed rats for 9 wk, CCl₄ treatment was discontinued; liver biopsies collected at that point revealed that fibrosis was present in all rats. Rats were then divided into two treatment groups and fed casein or glycine. The rate of regression of fibrosis was followed as depicted in Fig. 4. Fibrosis began to decline after 4 wk of recovery and was reduced by ~50% in both dietary groups within 20 wk. There was no difference in the rate of regression of fibrosis in rats fed casein or glycine.

**Effect of CCl₄ on plasma endotoxin levels.** Endotoxin is known to activate Kupffer cells and may mediate fibrosis that results from dietary choline deficiency (4, 36). To determine whether endotoxemia occurs during CCl₄-induced fibrosis, endotoxin was measured in platelet-rich plasma. Endotoxin was not detectable in plasma samples collected from the tail vein or in portal blood of oil-treated control rats. After 9 wk of CCl₄ treatment, endotoxin values in portal blood were 28 ± 4 pg/ml. Plasma endotoxin levels in rats given CCl₄ plus GdCl₃ or CCl₄ plus glycine were 24 ± 4 and 12 ± 6 pg/ml, respectively, and did not differ from rats treated with CCl₄ alone.

**Effect of glycine on p-nitrophenol metabolism.** Metabolic activation of CCl₄ to trichloromethyl radical is a prerequisite for hepatic injury; CYP2E1 is primarily

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**Fig. 3.** Average fibrosis scores. Rats were treated with CCl₄, and some were treated with GdCl₃ or fed a 5% glycine (Gly)-containing diet as described in MATERIALS AND METHODS. Rats were killed, and fibrosis was scored using the following method: 1 = thickened perivenular collagen and a few thin collagen septa; 2 = thin septa with incomplete bridging between portal regions; 3 = thin septa and extensive bridging; 4 = thickened septa with complete bridging of portal regions and nodular appearance. Values are means ± SE as analyzed with Kruskal-Wallace ANOVA on ranks. *P < 0.05 compared with control. #P < 0.05 compared with CCl₄ alone.
responsibility for this process (21). It has been shown previously that chronic administration of GdCl₃ does not alter CYP2E1 activity or protein levels induced by alcohol (24). To determine if the observed protective effects of glycine were due to decreased hepatic metabolism of CCl₄, the effect of feeding glycine on the hydroxylation of the CYP2E1-specific substrate p-nitrophenol was monitored after treatment with glycine or casein (control) and CCl₄ for 9 wk. The rate of microsomal p-nitrophenol hydroxylation after CCl₄ exposure was 0.7 ± 0.5 nmol·mg⁻¹·min⁻¹ and was not different from rates measured in vehicle controls. Furthermore, feeding rats glycine during CCl₄ administration did not significantly alter microsomal CYP2E1 activity (0.8 ± 0.3 nmol·mg⁻¹·min⁻¹).

**Effect of GdCl₃ and glycine on collagen mRNA levels.** To assess the effects of Kupffer cell destruction or inhibition on the rate of type I collagen synthesis, α1(I) collagen mRNA was quantified with an RNase protection assay. A representative assay is shown in Fig. 5. After 9 wk of CCl₄ exposure in rats fed chow, α1(I) collagen steady-state mRNA levels were increased fivefold. Treatment of rats with GdCl₃ during CCl₄ exposure blunted the increase in α1(I) collagen mRNA expression significantly, by ~70%. Glycine also blunted the induction of α1(I) collagen mRNA expression due to CCl₄ exposure by ~90%. Thus destruction or inhibition of Kupffer cell function markedly diminished new collagen synthesis in vivo.

**TGF-β protein levels.** Because TGF-β is involved in fibrosis, the effects of Kupffer cell destruction or inactivation on hepatic TGF-β1 protein levels was investigated. Exposure of rats to CCl₄ significantly increased total levels of TGF-β1 (active + latent) in frozen liver samples from 26.4 ± 1.2 pg/ml of liver homogenate in oil-treated controls to 55.9 ± 5.3 pg/ml. In contrast, TGF-β1 expression in livers from rats treated with GdCl₃ during CCl₄ exposure was not increased and was 28.2 ± 3.2 pg/ml of liver homogenate. Glycine was most effective and actually reduced TGF-β1 expression (2.8 ± 0.7 pg/ml).

**Effects of glycine and GdCl₃ on α-SMA expression.** It is well known that CCl₄ causes stellate cells to undergo phenotypic transformation to myofibroblast-like cells (44, 48); expression of α-SMA is characteristic of the new phenotype. Therefore, the effects of CCl₄, glycine, and GdCl₃ on stellate cell α-SMA expression were measured with immunohistochemistry. α-SMA was not detectable in liver sections from oil-treated control rats (Fig. 6A). As expected, CCl₄ treatment resulted in extensive α-SMA staining in portal areas, and the staining appeared to extend along collagen septa bridging portal areas; the average score for α-SMA staining in this group was 3.0 ± 0.2 (Fig. 6B). Treatment of rats with GdCl₃ during CCl₄ exposure largely blocked expression of α-SMA and resulted in a score of 1.2 ± 0.5 (Fig. 6C). Staining was prevented completely in rats fed a glycine-rich diet (Fig. 6D).

**DISCUSSION**

**Effect of GdCl₃ and glycine on Kupffer cells.** Previous experiments have shown that intravenous administration of GdCl₃ depletes Kupffer cells. For example, by using electron microscopy, Hardonk et al. (13) demonstrated that large Kupffer cells were no longer present 24 h after GdCl₃ treatment. Recently, Koop et al. (24) showed that GdCl₃ eliminated ~80% of a Kupffer cell-specific lectin (“Kupffer cell receptor”). Consistent with these findings, a 75% decrease in the number of ED1-positive Kupffer cells was observed after 9 wk of treatment with GdCl₃ in the present study.

Chronic treatment with GdCl₃ (24), or feeding glycine as performed in the present study, did not alter CYP2E1 activity; therefore, it is unlikely that these agents interfere with the bioactivation of CCl₄ to the toxic trichloromethyl metabolite. In a previous study, glycine prevented endotoxin-induced Kupffer cell acti-
vation and cytokine production by stimulating chloride influx and hyperpolarizing the cell membrane (20). Therefore, the protective effects of glycine against fibrosis most likely result from diminished Kupffer cell activity; however, a direct effect of glycine on stellate cell type I collagen production cannot be ruled out (see below).

**GdCl₃ and dietary glycine diminish hepatic fibrosis.** Fibrosis is a complex pathological process. In early stages, inflammation and necrosis occur, which may initiate hepatocyte regeneration and repair. Subsequently, there is increased accumulation of matrix proteins, and alterations in the normal architecture of the hepatic lobule occur. Impairment of metalloproteases, such as transin, that degrade the extracellular matrix is believed to contribute to fibrosis that results from CCl₄ exposure (15). In the present study, injury ranging from fibrosis to cirrhosis was observed (Fig. 2), confirming other work (32, 35, 45). Treatment of rats with GdCl₃ or glycine during CCl₄ exposure diminished collagen accumulation (Fig. 2, C and D) and markedly reduced the appearance of the bridging septa of matrix.

**Fig. 6. Effect of GdCl₃ and glycine on α-smooth muscle actin (α-SMA).** Expression of α-SMA, a marker of stellate cell activation, was assessed immunohistochemically in formalin-fixed liver sections. Representative photomicrographs of liver sections from rats treated with oil (A), CCl₄ (B), CCl₄ + GdCl₃ (C), and CCl₄ + glycine (D) are shown. Original magnification, ×60.

**Fig. 7. Schematic representation of the proposed effect of GdCl₃ and glycine on fibrosis.** CCl₄-induced fibrosis was associated with increased circulating levels of endotoxin. It is hypothesized that Kupffer cells are activated by endotoxin or free radicals released from hepatocytes after CCl₄ metabolism. Activated Kupffer cells release proinflammatory cytokines that cause necrosis and inflammation. In addition, production of transforming growth factor (TGF)-β1 stimulates proliferation and collagen production by stellate cells. Destruction of Kupffer cells with GdCl₃ and Kupffer cell inactivation with glycine most likely prevent the release of proinflammatory and profibrogenic cytokines, thus markedly reducing collagen accumulation and fibrosis. CYP450, cytochrome P-450.
proteins between portal areas that is characteristic of advanced fibrosis (Figs. 2 and 3). On the other hand, feeding glycine did not influence the rate of regression of fibrosis (Fig. 4), consistent with the hypothesis that glycine does not enhance collagen degradation and most likely does not affect metalloprotease activity. Therefore, it is likely that the protection against fibrosis observed in this study was due to decreased synthesis of matrix proteins. Indeed, the increase in a1(1) collagen mRNA expression caused by CCl4 was largely blocked by GdCl3 and glycine (Fig. 5). These results demonstrate that removal or inactivation of Kupffer cells in vivo has profound inhibitory effects on the pathogenesis of fibrosis.

Possible mechanism of Kupffer cell involvement in CCl4-induced hepatic fibrosis. It is well known that Kupffer cells release toxic free radicals and cytokines (6), and in vitro studies have demonstrated that Kupffer cells produce factors that activate stellate cells (48). As illustrated in Fig. 7, two important cytokines are PDGF and TGF-β1; there is evidence that they both play important roles in the proliferation and enhanced production of matrix proteins by stellate cells (8, 26). Although stellate cells are capable of producing TGF-β1 (27), recent studies indicate that increased expression of TGF-β1 mRNA in Kupffer cells isolated from rats with alcoholic fibrosis precedes expression in stellate cells (42). PDGF stimulates the release of retinol, an early event in stellate cell activation (9). Furthermore, PDGF receptor expression correlates with cell proliferation and collagen deposition in the early stages of CCl4-induced liver injury (46). Another profibrogenic cytokine, interleukin-6, has also been implicated in stellate cell activation (11, 42). Collagen production by cultured stellate cells has been shown to be stimulated by the addition of interleukin-6, and in vivo experiments have demonstrated that interleukin-6 mRNA levels in Kupffer cells are markedly increased, coincident with stellate cell transformation to myofibroblast-like cells.

In the present study, destruction of Kupffer cells with GdCl3 or inactivation by dietary supplementation with glycine blunted increases in TGF-β1 protein expression; fibrosis and hepatic collagen accumulation as a result of CCl4 treatment were similarly affected (Figs. 3 and 5). These results are consistent with the hypothesis that collagen production by stellate cells is potentially regulated in vivo by the early release of products from Kupffer cells.

Several studies suggest that endotoxin, a cell wall component of gram-negative bacteria and a potent Kupffer cell activator, is involved in liver pathogenesis (30). For example, Nolan and Leibowitz (31) demonstrated that treatment of rats with polymyxin B to eliminate gram-negative bacteria and endotoxin decreased injury resulting from acute CCl4 exposure. In addition, antibiotics blocked the development of hepatic fibrosis due to long-term feeding of a choline-deficient diet (36). Therefore, it is likely that endotoxin is a stimulus for Kupffer cell activation during CCl4-induced fibrosis. In support of this idea, increased circulating levels of endotoxin were observed in the rats treated with CCl4. However, endotoxin was not altered by GdCl3 or glycine. In response to increased circulating endotoxin levels, Kupffer cells may become activated to release mediators that stimulate the fibrotic process (see Fig. 7).

Finally, oxidative stress is also thought to play a role in Kupffer cell activation and fibrogenesis (5, 44). In fact, correlations have been made between hepatic peroxidative injury and enhanced Kupffer cell cytokine production during fibrosis resulting from bile duct ligation in rats (44). Although there is no direct evidence, oxidative stress cannot be ruled out as a possible stimulus for Kupffer cell activation during CCl4-induced fibrosis.

Conclusions. As illustrated in Fig. 7, it is hypothesized that chronic CCl4 exposure activates Kupffer cells, possibly via the release of radicals or by causing mild endotoxemia. The present study demonstrates that destruction of Kupffer cells with GdCl3, as well as Kupffer cell inactivation with dietary glycine, diminishes stellate cell α-SMA expression and collagen production, most likely by preventing the release of profibrogenic cytokines from Kupffer cells. Alternatively, previous studies (25, 29, 40) suggested that early inflammation contributes to the later development of fibrosis. Because GdCl3 has been shown to prevent tissue injury and inflammation resulting from acute CCl4 exposure (7), it is also possible that Kupffer cell destruction diminishes fibrosis by blunting early hepatic necrosis and inflammation. Whereas conditioned medium and coculture experiments predict a role for Kupffer cells in fibrogenesis (12, 26, 38, 48), this study provides direct in vivo evidence that Kupffer cells are involved in this pathology. Thus agents that selectively block Kupffer cell activation may provide effective therapy against the progression of fibrosis in humans.

REFERENCES

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