Increased Procollagen mRNA Levels in Carbon Tetrachloride-induced Liver Fibrosis in Rats*

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Carbon tetrachloride-induced liver damage is a well-characterized experimental model for studying liver fibrosis. We used this model to examine α1(I), α1(III), and α1(IV) procollagen mRNA levels during the development of liver fibrosis. Rats were given 0.5 ml of carbon tetrachloride/kg of body weight for 1–6 weeks. The liver tissue was assayed for collagen content by measuring total hydroxyproline content. Specific increases in procollagen mRNAs were assayed by slot blot hybridization. There was a significant increase in hydroxyproline content of liver tissue following 3 weeks of carbon tetrachloride treatment. The increase in tissue collagen content correlated with an increase in α1(I) procollagen mRNA levels. At 5 and 6 weeks of treatment, there was an increase in α1(III) procollagen mRNA levels. α1(IV) procollagen levels increased slightly with five injections of carbon tetrachloride treatment. These results suggest that specific increases in procollagen mRNAs in liver fibrosis parallel, but do not precede, increases in tissue collagen content.

Normal liver contains low levels of collagen types I, III, and IV (1, 2). Type I and III collagen are present in approximately equal amounts and constitute from 0.5 to 2% of total protein in the normal liver (3, 4). In fibrotic and cirrhotic liver, the total collagen content increases. It has been reported that type III collagen increases in the initial fibrotic process, whereas type I collagen predominates in advanced liver fibrosis and cirrhosis (5-7). Type IV collagen is a nonfibrillar collagen localized to basement membranes. However, in liver, type IV collagen is found in the subendothelial space (Disse) of sinusoids, which does not have a basement membrane. Type IV collagen also increases in liver fibrosis and cirrhosis (8).

Hepatocytes, or parenchymal cells, constitute the majority of the cells in the liver. The presence of prolyl hydroxylase activity in these cells suggests they may be involved in the synthesis of collagen in liver. The localization by immunohistochemical staining of intracellular type I procollagen to injured hepatocytes also suggests that these cells can synthesize collagen (8). Endothelial cells lining the sinusoids are involved in type IV procollagen synthesis (9). In fibrosis and cirrhosis, the additional recruitment of fibroblasts contributes to the cell population which is actively synthesizing collagen.

Experimental fibrosis and cirrhosis can be produced by chronic carbon tetrachloride intoxication (10, 11). A single dose of carbon tetrachloride leads to centrilobular necrosis and steatosis (12). Prolonged administration of carbon tetrachloride leads to fibrosis, cirrhosis, and hepatic carcinoma (13, 14). The mechanism of hepatic injury by carbon tetrachloride is mediated by a free radical metabolite which is involved in disruption of cellular membranes and organelles. The fibrosis which follows chronic carbon tetrachloride intoxication is probably part of a complex inflammatory, immune, and repair response (15). Extensive disruption of the organization of the parenchyma followed by increased interstitial collagen in the extracellular matrix is a characteristic of the early fibrotic response to carbon tetrachloride injury.

In carbon tetrachloride-induced liver fibrosis, increases in the levels of prolyl hydroxylase, an enzyme involved in the post-translational hydroxylation of procollagen, have been previously reported (16). The increases in the activity of this enzyme preceded the increases in total hydroxyproline content of the fibrotic livers. To further characterize the temporal sequence of biosynthetic events associated with collagen synthesis in liver fibrosis, we measured mRNA levels coding for type I, III, and IV procollagen in the early stages of carbon tetrachloride-induced fibrosis in rat livers. The results described in this manuscript indicate that carbon tetrachloride-induced liver fibrosis in rats is characterized by separate temporal increases in the mRNA coding for these three procollagens. Although an increase in α1(I) procollagen mRNA levels paralleled an increase in hydroxyproline content of the liver, a significant elevation of α1(III) procollagen and α1(IV) procollagen mRNA levels was apparent at a slightly later time point.

EXPERIMENTAL PROCEDURES

Materials

Male Sprague-Dawley rats were obtained from Charles River, Inc. Nitrocellulose filters and the slot blot manifold were purchased from Schleicher and Schuell. All radiolabeled compounds were provided by ICN. Most reagents were purchased from International Biotechnologies, Inc. and were of ultrapure or equivalent grade. X-ray film (Cronex R) was purchased from Du Pont and Kodak. X-Omatic cassettes with regular intensifying screens were used for autoradiography. The β scanner was obtained from Automated Microbiology Systems (San Diego).

1 The term procollagen refers to the newly synthesized, partially processed precursor of the extracellular matrix protein, collagen.
Procollagen mRNA in Fibrosis

Methods

Carbon Tetrachloride Treatment—Rats were maintained on Purina Rat Chow and water ad libitum. Experimental rats received twice-weekly intraperitoneal injections of carbon tetrachloride/mineral oil (1:1) at a dose of 1 ml/kg of body weight (16). Rats were killed 4 days after their last injection. Groups of eight rats each receiving 1, 2, 4, 6, 8, 10, and 12 injections were killed with 2-5 age-matched controls. For biochemical and statistical analysis, control animals from all time points were grouped together.

Tissue Sampling—Body and whole liver weights were recorded at autopsy. Adhesions of the liver to the omentum, peritoneum, and diaphragm were noted when present. Gross appearance of the liver was recorded. The middle lobe of the liver was removed and a specimen taken for histology. Histological specimens were fixed in Carson's buffered formalin, mounted, and stained with hematoxylin and eosin, Masson's trichrome, or Verhoeff and Van Geissen stain. The remaining tissue from the middle lobe was diced finely with a scalpel and divided into samples for protein and hydroxyproline assay, DNA assay, and RNA extraction. All specimens were stored at -70 °C.

RNA Extraction—RNA was extracted from liver tissue using a guanidinium isothiocyanate/cesium chloride method described by Gilsin et al. (17). 0.5 g of tissue was finely diced while frozen and the rinsed nitrocellulose filters were placed in Kodak in 5 volumes of 4 M guanidinium isothiocyanate, 5 mM sodium citrate, 0.1 M β-mercaptoethanol, 0.5% sarkosyl (ICN). CaCl₂ (0.4 g/ml) was dissolved in the homogenate, then was layered on a cushion of 5.7 M CaCl₂, 0.1 M EDTA, and centrifuged at 140,000 × g at 20 °C for 16 h. The supernatant was discarded, and the pellet, which was suspended in distilled water, was pelleted with equal volume of 1% CTAB/2.2 M NaCl, 0.1 M EDTA. The pellets were resuspended in 10 mM Tris, pH 7.4, 5 mM EDTA, 1% SDS. The solution was extracted with phenol/chloroform/isooamyl alcohol (50:50:1) and ethanol-pre-cipitated. The concentration and purity of each preparation was determined by measuring the optical density at 260 and 280 nm.

Slot Blotting of RNA—For each RNA sample, 150-μl mixtures containing 0, 100, 200, 300, 400, and 500 ng of liver RNA were prepared on ice. These mixtures contained 75 μl of 10 × SSC-formaldehyde (1:1), 10 μl of tRNA (20 μg/ml), liver RNA, and water to bring the volume to 150 μl. The samples were heated at 65 °C for 15 min, placed on ice for 10 min, and immediately applied to the nitrocellulose. Samples containing 100 ng of yeast tRNA (Sigma) and 100 ng of reticulocyte lysate RNA were applied to each filter to determine nonspecific binding of the probes. Reticulocyte lysate RNA was obtained by guanidinium isothiocyanate extraction of rabbit reticulocyte lysate (Promega).

Prehybridization filters were soaked in 10 × SSC for 2 h at room temperature with gentle agitation. The RNA preparations were blotted onto nitrocellulose filters under vacuum using the Minifold slot blot apparatus. Each slot was rinsed three times with 200 μl of 10 × SSC. The filters were air dried and then baked at 80 °C under vacuum for 2 h. Filters were stored at room temperature in sealed plastic bags.

Hybridization of Slot Blotted Filters—The nitrocellulose filters with slot blotted RNA were prehybridized in sealed plastic bags containing 50 ml of 1% (v/v) sarkosyl, 3 × SSC containing the 32P-labeled RNA probe added. The sealed bags were incubated overnight at 65 °C. The prehybridized filters were washed at 65 °C. Two 30-min washes in 250 ml of 0.5% sarkosyl, 3 × SSC were followed by two 30-min washes in 3 × SSC. The filters were air dried and then placed in sealed plastic bags.

 Autoradiograms were photographed in Kodak X-Omat cassette with intensifying screens and Du Pont Cronex x-ray film. Cassette were kept at -70 °C for 1 h, brought to room temperature for 15 min, and the film was processed in a Kodak automatic processor. Autoradiograms were used to visually assess hybridization of the probes to the RNA on the filters.

Protein-Hydroxyproline Assay—One or two control animals were selected at random for each injection point. Samples of liver tissue weighing 0.5 g were homogenized in 5 volumes of water. The homogenates were mixed in HCl by the addition of an equal volume of 12 N HCl. Samples were hydrolyzed for 4 h at 116 °C. Aliquots of the hydrolysate were stored at -20 °C. Duplicates of hydrolysate were assayed for total hydroxyproline as described by Kivirikko et al. (18). Total protein was assayed using a ninhydrin (19) assay and compared to a leucine amino acid standard.

DNA Assay—Samples of liver tissue weighing 0.2 g were homoge-

ized in 4 ml of 0.5 N perchloric acid. Deoxyribonucleic acid content was assayed by a colorimetric assay as described by Burton (20). Two dilutions of each sample were assayed in duplicate. The concentrations of the DNA samples were determined using a standard curve prepared from five dilutions of herring sperm DNA.

Preparation of [32P]-Labeled RNA Probes—Single-stranded RNA riboprobes labeled with [32P]UTP were propagated from cDNA templates for α1 type I (kindly provided by Dr. D. J. Frocock, Jefferson Medical College, Philadelphia), α1 type III (kindly provided by Dr. J. C. Myers, Connective Tissue Research Institute, Philadelphia), α1 type IV (procollagen mRNAs, and γ-actin mRNA (kindly provided by Dr. P. Gunnung, UCLA). The riboprobe transcripts were propagated according to protocol 1 provided by Promega Biotech using high specific activity [32P]UTP (3000 cpm/mM UTP, ICN).

1 μg of linearized recombinant DNA was used in each reaction. For α1 (I) procollagen, the 1.5-kb EcoRI fragment of Hf 677 (21) subcloned into pSP 65 was linearized with Aca1. For α1 (III) procollagen, a 700-bp PstI fragment of RJS (22) subcloned into pSP64 was linearized with BamHI. The α1(IV) procollagen probes were made from a 2-kb EcoRI, BamHI fragment of HT-21 (23) subcloned into pSP 65 linearized with BamHI primed with 32P-labeled γ-actin DNA. For α1 type II (procollagen) and γ-actin DNA (kindly provided by Dr. P. Gunnung, UCLA). The riboprobe transcripts were propagated using protocol 1 provided by Promega Biotech using high specific activity [32P]UTP (3000 cpm/mM UTP, ICN).

The ratios of α1(I) and α1(III) procollagen mRNAs were determined by densitometric scanning of autoradiograms at several exposure times. Ratios were adjusted for probe size and specific activity. Both

1 The abbreviations used are: kb, kilobase; MOPS, 4-morpholine-ethanesulfonic acid; 1 × SSC, 0.15 M NaCl, 0.01 M sodium citrate; 1 × Denhardt's, 0.02% bovine serum albumin, 0.02% polyvinylpyrrolidone, 0.02% Ficoll; SDS, sodium dodecyl sulfate.
clones cover approximately the same regions of the mRNA (most of the carboxyl propeptide coding region and about 800 base pairs of triple helical coding region). Base sequence complexity and the kinetics of complementary strand reassociation, therefore, can be assumed to be approximately the same for both probes.

For determination of ribosomal RNA levels, a 7.3-kb clone (pA4) containing 5.8 and 28 S genomic DNA and a 5.7-kb clone containing 18 S genomic DNA (772B) were kindly provided by Dr. J. E. Sylvestre, University of Pennsylvania, Philadelphia (26, 27). 0.5 µg of DNA from each clone was pooled and labeled by nick translation. The probe was hybridized to a serial dilution of 50–500 ng of slot blotted total RNA (1, 2, 10-week control samples, two 10-week, two 12-week experimental RNA samples) as described above. The amount of probe bound was quantitated using a computerized β scanner.

Statistical Analysis—A two-tailed Student's t test with p ≤ 0.05 was used to determine statistical significance (28). Two p values reported above p < 0.05 but below p < 0.10 are significant by a one-tailed Student's t test, but are considered as below statistical significance.

RESULTS

Carbon Tetrachloride-induced Liver Fibrosis—Six weeks of carbon tetrachloride treatment resulted in the progressive development of steatosis and fibrosis in the livers of all experimental animals. Visual examination of the livers revealed fatty foci following a single dose of carbon tetrachloride. Fat infiltration of the liver was progressive with the duration of the treatment, and in the later phases (four or more injections), extensive thickening of the liver edge, fibrosis, and adhesions were observed. These observations were supplemented by histological examination of liver sections at each injection point. Fig. 1 shows extensive disruption of the parenchymal organization at 6 weeks of carbon tetrachloride treatment. Trichrome staining confirmed the increased collagen content of the stroma (not shown).

Carbon tetrachloride treatment resulted in a significant reduction in the body weight of the experimental animals compared to the control animals (Table I). It has been shown that starvation can selectively inhibit collagen metabolism in various tissues (29). In carbon tetrachloride-induced liver fibrosis, the difference in body weights of the control and experimental animals did not inhibit specific increases in the hydroxyproline content of fibrotic livers (Table II).

There was no increase in the relative hydroxyproline content of control livers during the time course of the experiment. The hydroxyproline content of control rat livers at week one was 2.85 ± 0.27 µg/mg protein (N = 4) and at weeks five and six was 2.49 ± 0.25 µg/mg protein (N = 3). A significant elevation in the hydroxyproline content of experimental rat livers was obtained after six injections (Table II). With subsequent injections, the hydroxyproline content of the experimental livers increased and stabilized at about 8 µg/mg protein. There was no significant alteration in total DNA content of the tissue (Table II), suggesting that the significant increase in the collagen content of the parenchyma was not the result of an increase in the number of cells synthesizing collagen.

Rat Liver Procollagen mRNA—High specific activity (10⁶ cpm/µg) single-stranded riboprobes were used to measure procollagen mRNA levels in rat liver. Fig. 2, lane A, shows nick-translated al(I) procollagen probe hybridized to poly(A)+ RNA isolated from cultured human skin fibroblasts. Lane B shows that hybridization of the same RNA with an al(I) procollagen riboprobe gave identical results. Similar results were obtained using total human fibroblast RNA. Hybridization of rat liver total or poly(A)+ RNA with an al(I) procollagen riboprobe showed a predominant band at 4.8 kb (Fig. 1C). Hybridization of rat liver RNA with al(III) procollagen riboprobe also showed a predominant single band of approximately 4.8 kb (lane C). Since multiple transcripts for both al(I) procollagen and al(III) procollagen mRNA have been reported (21, 22), preferential usage of a specific polyadenylation site in rat liver may account for the predominant single band for both procollagen messages.

Northern blot analysis of RNA isolated from reticuloocyte lysate, which contains significant amounts of ribosomal RNA, showed no detectable hybridization with either al(I) or al(III) procollagen probes, indicating that the strongly hybridizing 4.8-kb bands were not due to hybridization to ribosomal RNA.

Quantitation of mRNA Levels—Serial dilutions of total RNA from individual control and experimental rat livers were probed with high specific activity riboprobes for al(I), al(III), al(IV) procollagen and γ-actin. The filters were hybridized and washed as described under "Experimental Procedures." The amount of probe bound to nitrocellulose filters was

![Fig. 1. Histological staining of rat liver specimens (x 100).](image)

**Table I**

| Weeks of treatment | Body weights of animals |
|-------------------|------------------------|
|                   | Experimental | Control |
| g, mean ± S.E.    | n           | g, mean ± S.E. | n |
| 1                 | 186.9 ± 5.6  | 10        | 202.5 ± 3.9  | 5 |
| 2                 | 228.0 ± 9.7* | 5         | 257.8 ± 3.6  | 5 |
| 6                 | 320.8 ± 4.0* | 5         | 407.4 ± 13.2 | 5 |

* Indicates significant difference from control, p < 0.05.
**TABLE II**

Hydroxyproline and DNA content of rat liver

| Carbon tetrachloride injections | µg hydroxyproline/mg protein | µg DNA/mg tissue |
|--------------------------------|-----------------------------|-----------------|
| n                              | X ± S.E.                    | X ± S.E.        |
| 0                              | 11                          | 8               |
| 1                              | 8                           | 5               |
| 2                              | 8                           | 5               |
| 4                              | 7                           | 5               |
| 6                              | 6                           | 5               |
| 8                              | 8                           | ND              |
| 10                             | 8                           | ND              |
| 12                             | 8                           | 5               |

*p < 0.001.

*ND, not determined.

*p < 0.01.

**FIG. 2. Northern blot analysis of rat liver RNA.** A, nick-translated probe for α1(I) procollagen hybridized to poly(A)*+ RNA from human cultured skin fibroblast. B, riboprobe for α1(I) procollagen (carboxyl-propeptide region) hybridized to poly(A)*+ RNA isolated from human fibroblasts. C, riboprobe for α1(I) procollagen (carboxyl-propeptide region) hybridized to rat liver RNA. D, riboprobe for α1(III) procollagen (carboxyl-propeptide region) hybridized to rat liver RNA.

Quantitated by a computerized Ambis β scanner. Fig. 3 shows a typical autoradiogram and the computer-generated visualization and quantitation of the radioactivity bound to the filter. The cpm/slot were determined by computerized integration of the area under each peak. Under the conditions of hybridization and washing, no nonspecific binding of any of the probes was detected to slots containing yeast tRNA. However, some nonspecific binding with all four riboprobes was detected to slots containing rabbit reticulocyte RNA, suggesting that under these slot blotting hybridization conditions, some nonspecific hybridization to ribosomal RNA was present.

To determine if any changes in ribosomal RNA content could affect the results, total RNA from control (1-, 2-, 10-week) and experimental (two 10-week, two 12-week) rat livers were probed with genomic clones for 18, 5.8, and 28 S RNA. There was no significant difference in ribosomal RNA content of the control (50.83 ± 5.07 cpm/ng RNA, N = 3) or the experimental (56.00 ± 2.36 cpm/ng RNA, N = 4) rat livers.

**Procollagen mRNA Levels in Liver Fibrosis—** We followed the course of carbon tetrachloride-induced liver fibrosis from a single injection to 12 injections of carbon tetrachloride to determine the correlation between the extent of fibrosis and the relative levels of procollagen mRNAs. Fig. 4 shows a significant elevation of the α1(I) procollagen/γ-actin mRNA ratio after six treatments of carbon tetrachloride. This increase in α1(I) procollagen mRNA is coincident with a statistical elevation of hydroxyproline content of the liver (Table II). These results suggest that a moderate elevation of α1(I) procollagen mRNA is observed with six or more injections of carbon tetrachloride. The elevation of α1(I) procollagen mRNA is coincident with an increase in total collagen content of the liver, suggesting that the initial increase in liver collagen content is at least in part a result of increased type I collagen synthesis.

An increase in α1(III) procollagen/γ-actin mRNA was observed with subsequent carbon tetrachloride treatments. Compared to control values, a greater relative increase in α1(III) procollagen/γ-actin mRNA was observed (Fig. 5). At 12 injections of carbon tetrachloride, the α1(III) procollagen/γ-actin mRNA ratio was increased to 140% of the control value, whereas the increase in α1(I) procollagen/γ-actin mRNA was about 115% of control. However, comparisons of the relative increases in type I and III procollagen mRNA at 12 injections gave a *p* = 0.073, just below statistical significance using a two-tailed *t* test.

The type IV procollagen/γ-actin mRNA ratio was not consistently elevated in parallel with increased dosages of carbon tetrachloride (Fig. 6), suggesting a difference in the regulation of type IV procollagen mRNA compared with interstitial procollagen mRNA in liver fibrosis.

Variability in actin mRNA levels probably explains some
of the fluctuations in procollagen/actin mRNA ratio. It has been reported that a single injection of carbon tetrachloride results in increased actin mRNA levels (30). These results are consistent with an increase in cell mitotic activity, associated with repair of damaged cells, observed 3 days following a single dose of carbon tetrachloride. The drop in the procollagen/γ-actin mRNA ratio following a single dose of carbon tetrachloride is presumably a result of this increased actin mRNA synthesis.

However, the progressive increase in α1(I) procollagen/ and α1(III) procollagen/γ-actin mRNA ratios is not likely to be solely the result of a progressive decrease in actin mRNA levels because the increase in interstitial procollagen mRNA levels correlated with an increase in hydroxyproline content of the liver.

Type I/Type III Procollagen mRNA Ratio—To determine how mRNA levels correlate with published values for rat liver type I and type III collagen content, we measured the procollagen mRNA ratio of rat liver using α1(I) and α1(III) procollagen nick-translated probes. Since riboprobes do not always give full-length transcripts, it was necessary to use nick-translated probes to determine absolute mRNA ratios. The α1(I) and α1(III) probes we used were approximately similar in size and sequence homology. Under slot blot hybridization conditions, no nonspecific binding of nick-translated probes to either yeast or reticulocyte RNA was observed. Therefore, the ratio of α1(I)/α1(III) procollagen probe bound should reflect the actual ratio of these mRNAs in the tissue. The ratio of α1(I)/α1(III) procollagen mRNA in control tissue was 0.61 ± 0.03 (N = 3). After adjusting for the protein stoichiometry of two α1 chains in type I collagen and three α1 chains in type III collagen, the ratio of α1(I)/α1(III) procollagen mRNA is 0.92. The results indicate that the potential for synthesis of these collagens, as reflected by mRNA levels, is about equal. These results are consistent with published results, which indicate approximately equal amounts of type I and type III collagen in normal rat liver (1).

FIG. 4. Type I procollagen mRNA levels during carbon tetrachloride-induced liver fibrosis. The ratio of α1(I) procollagen/γ-actin mRNA was determined as described under "Experimental Procedures." The results are expressed as a percent of the mean control α1(I) procollagen/γ-actin mRNA ratio (N = 14). The data are reported as mean ± standard error. The numbers in parentheses are the number of animals examined at each point. For statistical analysis, the mean control ratio was set to 100% and the S.E. expressed as a percent of the mean. (100% ± 2.45%) \( \Delta p < 0.10 \) (not statistically significant by a two-tailed t-test); \( *p < 0.02; **p < 0.001 \).

FIG. 5. Type III procollagen mRNA levels during carbon tetrachloride-induced liver fibrosis. The ratio of α1(III) procollagen/γ-actin mRNA was determined as described under "Experimental Procedures." The results are expressed as a percent of the mean control α1(III) procollagen/γ-actin mRNA ratio (N = 14). The data are reported as mean ± standard error. The numbers in parentheses are the number of animals examined at each point. For statistical analysis, the mean control ratio was set to 100% and the S.E. expressed as a percent of the mean (100 ± 5.76%). \( *p < 0.05; **p < 0.01 \).

DISCUSSION

It has been shown that mRNA levels for procollagen parallel procollagen protein levels in skin fibroblasts grown in cell culture (31). We examined procollagen mRNA levels in normal rat liver and in rat liver during early stages of experimental liver fibrosis to determine how mRNA levels correlated with total tissue collagen content. We measured procollagen mRNA levels relative to γ-actin mRNA levels to determine specific increases in procollagen mRNA relative to the mRNA coding for a noncollagenous protein.

Our results suggest that mRNA levels for interstitial procollagens parallel the collagen content of rat liver tissue. Significant increases in the collagen content of the liver, as determined by hydroxyproline content, occurred simultaneously with a significant increase in α1(I) procollagen mRNA levels. These results would imply that the first significant increase in the collagen content of fibrotic liver is due, at least in part, to increases in type I collagen. Although decreased degradation of collagen may contribute to the increased collagen content of the liver, our results indicate that increased synthesis of procollagen is a major contributing factor to the increased collagen content of fibrotic liver.

The initial selective increases in steady-state levels of in-
terstitial procollagen mRNAs are either the result of increased procollagen synthesis by hepatocytes (8) or by a population of other cells in the stroma (32). Our results show no significant increase in the DNA content of the liver, suggesting a per cell increase in procollagen synthesis. The increased procollagen mRNA steady-state levels we have observed may be the result of increased gene expression by liver stromal cells. However, a selective decrease in the degradation of procollagen mRNAs (33) cannot be ruled out.

Examination of rat livers following a single dose of carbon tetrachloride revealed foci of steatosis. With progressive dosage, there appeared extensive steatosis, discoloration and thickening of the liver, fibrosis, and adhesions to the diaphragm and omentum. The extent of these pathological changes was progressive with the time of carbon tetrachloride dosage and in some animals preceded statistical elevations in hydroxyproline content and procollagen mRNA levels. Histological examination of the livers from carbon tetrachloride-treated rats revealed extensive disruption of parenchymal architecture. The most extensive fibrosis was evident at 6 weeks of treatment. The fibrosis was detectable with both elevated liver hydroxyproline content and procollagen mRNA levels. In general, increased steady-state levels of type I and III procollagen mRNAs, commencing with six doses of carbon tetrachloride, paralleled increased hydroxyproline content and increased pathological changes in the liver. These results suggest that mRNA levels of procollagen may be useful indicators of early fibrosis of the liver. However, liver tissue has the ability to regenerate and repair itself following limited damage (13). Because of the regenerative ability of the liver, it is possible that procollagen/γ-actin mRNA ratios may not reflect the earliest increase in procollagen mRNA levels until extensive carbon tetrachloride damage inhibits cellular repair processes, and subsequent actin synthesis, in the liver.

Using this same model of experimental liver fibrosis, Tak-euchi and Prockop (16) have reported that significant increases in prolyl hydroxylase activity precede significant increases in hydroxyproline content of fibrotic liver. Their results indicate that increased prolyl hydroxylase activity is detectable prior to increased hydroxyproline in the fibrotic liver. We found that specific increases in procollagen mRNA paralleled but do not precede increased hydroxyproline content of fibrotic livers. These results imply that specific increases in procollagen mRNA levels do not precede increased prolyl hydroxylase activity in fibrosis of the liver.

Type III procollagen carboxyl propeptide antigen has been suggested as a useful indicator of liver fibrosis (34). In the carbon tetrachloride-treated rat liver, the relative increase in α1(III) procollagen/γ-actin mRNA was greater than the relative increase in α1(I)/γ-actin mRNA ratio. Our findings suggest that increases in type III procollagen carboxyl propeptide antigen in the early stages of liver fibrosis or recurrent phases of the disease will be as a result of increased synthesis of type III procollagen.

However, in our model, type I procollagen mRNA is elevated at a slightly earlier point in the fibrosis than type III procollagen mRNA. It has been reported that with carbon tetrachloride-induced liver fibrosis in rats, type I and III collagen are equally increased (1). Therefore, the initial increase in type I procollagen mRNA may parallel in synthetic potential the ensuing, albeit relatively greater, increase in type III mRNA.

It is interesting to note that type I, III, and IV procollagen mRNA levels were not increased simultaneously (Figs. 4, 5, and 6), suggesting a difference in the regulation of the steady-state levels of these mRNAs in fibrotic liver. Differential regulation of type I and III procollagen synthesis has been indicated in the decrease in type III relative to type I collagen during fetal development of the dermis and during the progressive maturation of granulation tissue to scar tissue (35). Our results suggest that the relative steady-state levels of type I, III, and IV procollagen mRNAs are independently regulated during the development of liver fibrosis.

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Procollagen mRNA in Fibrosis

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