Halofuginone to Prevent and Treat Thioacetamide-Induced Liver Fibrosis in Rats

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Hepatic fibrosis is associated with activation of hepatic stellate cells (HSC), the major source of the extracellular matrix (ECM) proteins. The predominant ECM protein synthesized by the HSC is collagen type I. We evaluated the effect of halofuginone—an inhibitor of collagen synthesis—on thioacetamide (TAA)-induced liver fibrosis in rats. In the control rats the HSC did not express smooth muscle actin, collagen type I gene, or tissue inhibitor of metalloproteinases-2 (TIMP-2), suggesting that they were in their quiescent state. When treated with TAA, the livers displayed large fibrous septa, which were populated by smooth muscle actin–positive cells expressing high levels of the collagen α1(I) gene and containing high levels of TIMP-2, all of which are characteristic of advanced fibrosis. Halofuginone given orally before fibrosis induction prevented the activation of most of the stellate cells and the remaining cells expressed low levels of collagen α1(I) gene, resulting in low levels of collagen. The level of TIMP-2 was almost the same as in the control livers. When given to rats with established fibrosis, halofuginone caused almost complete resolution of the fibrotic condition. The levels of collagen, collagen α1(I) gene expression, TIMP-2 content, and smooth muscle actin–positive cells were as in the control rats. Halofuginone inhibited the proliferation of other cell types of the fibrotic liver *in vivo* and inhibited collagen production and collagen α1(I) gene expression in the SV40-immortalized rat HSC-T6 cells *in vitro*. These results suggest that halofuginone may become an effective and novel mode of therapy in the treatment of liver fibrosis. (HEPATOLOGY 2001;33:379-386.)

Hepatic fibrosis represents the response of the liver to diverse chronic insults such as parasitic disease, chronic viral infection (hepatitis B and C), immunologic attack (autoimmune hepatitis), hereditary metal overload, toxic damage, etc. Because of the worldwide prevalence of these insults, liver fibrosis is common and is associated with significant morbidity and mortality. The elucidation of the cellular and molecular mechanisms responsible for the development and progression of the liver fibrosis[1] has provided a sound basis for development of pharmacologic strategies able to modulate the course of the disease.[2,3] The cellular mechanisms of hepatic fibrosis are shared among the various insults and, in many aspects, mirrors the scarring and wound-healing processes of other tissues.[4,5] Hepatic fibrosis, regardless of the cause, is characterized by an increase in extracellular matrix (ECM) constituents, although the relative distribution within the liver lobule varies with the site and nature of the insult.[3] In the injured liver, the hepatic stellate cells (HSC) lying in the space of Disse beneath the endothelial cell layer, constitute the major source of the ECM proteins.[4,6,7] These cells are usually quiescent, with a low proliferation rate; on activation, probably because of hepatocyte injury,[8] they differentiate into myofibroblast-like cells, with high proliferative capacity.[7] The predominant ECM protein synthesized by the HSC in fibrosis is collagen type I, which increases from approximately 2% of total proteins in a normal human liver to 10% to 30% in a cirrhotic liver.[9-11] In experimental models of fibrosis of various types, the increased deposition of type I collagen results primarily from increased transcription of the type I collagen genes.[12-14] In hepatic fibrosis, increases in the gene expression of other types of collagens such as III and IV[15,16] and other matrix proteins[17,18] have also been reported. In addition to being caused by increased ECM synthesis, liver fibrosis may also result from relative imbalance between production and degradation of matrix proteins. It has been shown that activated stellate cells constitute the source of various collagenases and tissue inhibitors of metalloproteinases (TIMPs), which are necessary for the ECM remodeling.[5,19,20] The lack of a specific inhibitor(s) of any component of the ECM in general and of collagen type I in particular, limits the success of prevention and treatment of hepatic fibrosis, although numerous agents have been tried.[14,21-24]

Halofuginone is a well-known inhibitor of collagen type I synthesis,[25] which has been reported to inhibit the gene expression of collagen type α1(I) but not of type α2(I), type II,[26] type III,[27] or type X (M. Pines, unpublished data). In culture, halofuginone has been found to attenuate collagen α1(I) gene expression and collagen production by murine, avian, and human skin fibroblasts derived from either scleroderma or chronic graft-versus-host disease (cGvHD) patients.[28] In animal models in which fibrosis was induced, halofuginone prevented the increase in collagen synthesis and collagen α1(I)

Abbreviations: ECM, extracellular matrix; HSC, stellate cells; TIMP-2, tissue inhibitor of metalloproteinases-2; cGvHD, chronic graft-versus-host disease; TSK, tight skin mouse; TAA, thioacetamide; PCNA, proliferation cell nuclear antigen; PBS, phosphate-buffered saline.

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gene expression. These models included mice afflicted with cGvHD,[29] rats with pulmonary fibrosis after bleomycin treatment,[30] and rats developing adhesions after surgery in tendons,[31] the abdomen,[32] and uterine horns.[33] In liver, halofuginone prevented collagen type I gene expression in dimethylnitrosamine-induced cirrhosis in rats.[34] In addition to its ability to prevent fibrosis by inhibiting the collagen α1(I) gene expression, halofuginone treatment caused an attenuation of the collagen already deposited in skin. This may be possible by altering the balance between synthesis and degradation of collagen type I as shown in tight skin (Tsk+) mice[29] and in a cGvHD patient.[35]

The goal of the present study was to evaluate the possibility of using halofuginone both to prevent and to treat hepatic fibrosis. Our results show that by inhibiting collagen synthesis, halofuginone can affect stellate cell activation, accelerate collagen degradation, and cause resolution of the fibrotic lesion, and could thus become a novel therapy for liver fibrosis.

MATERIALS AND METHODS

Materials. A 1,600-bp rat collagen α1(I) probe was a generous gift from B.E. Kream, University of Connecticut, Farmington, CT. Halofuginone bromhydrate was obtained from Collgard Biopharmaceuticals Ltd (Tel Aviv, Israel). Thioacetamide (TAA) was obtained from Sigma Chemicals Co. (St. Louis, MO). Smooth muscle actin monoclonal antibodies, used at 1:200 dilution, and proliferation cell nuclear antigen (PCNA) were from Dako A/S (Glostrup, Denmark). TIMP-2 polyclonal antibodies, corresponding to the carboxyl terminus of human TIMP-2, used at 1:50 dilution, were from Santa Cruz Biotechnology, Inc, (Santa Cruz, CA). As a second antibody, the Histomouse SP kit was used (Zymed Laboratories Inc, South San Francisco, CA).

Animals. Male Wistar rats (200-250 g) were kept in the animal breeding house of the Wolfson Medical Center and fed Purina rodent chow *ad libitum*. All animals received humane care during the study, under a protocol that was in accordance with institutional guidelines. Cirrhosis was induced in rats by intraperitoneal administration of TAA 200 mg/kg twice weekly for 12 weeks, as described previously.[36] Such long-term administration of TAA results in characteristic lesions of micronodular cirrhosis in the liver.[37,38] Halofuginone was given in the diet at a concentration of 5 ppm, which had previously been found to be effective in preventing abdominal and uterine horn adhesion formation in rats.[32,33] Hydroxyproline was analyzed by an amino acid analyzer after hydrolysis.[39]

Preparation of Sections, **In Situ** *Hybridization, and Immunohistochemistry.* Liver samples were collected into phosphate-buffered saline (PBS) and fixed overnight in 4% paraformaldehyde in PBS at 4°C. Serial 5-μm sections were prepared after the samples had been dehydrated in graded ethanol solutions, cleared in chloroform, and embedded in Paraplast. Differential staining of collagenous and noncollagenous proteins was performed with 0.1% sirius red, with 0.1% fast green as a counter stain, in saturated picric acid. By this procedure collagen is stained red.[40] For hybridization, the sections were deparaffinized in xylene, rehydrated through a graded series of ethanol solutions, rinsed in distilled water (5 minutes), and incubated in 2 × sodium saline citrate at 70°C for 30 minutes. The sections were then rinsed in distilled water and treated with pronase (0.125 mg/mL in 50 mmol/L Tris-HCl, 5 mmol/L ethylenediaminetetraacetic acid, pH 7.5) for 10 minutes. After digestion, the slides were rinsed with distilled water, post-fixed in 10% formalin in PBS, blocked in 0.2% glycine, rinsed in distilled water, rapidly dehydrated through graded ethanol solutions, and air dried for several hours. Before hybridization, the 1,600-bp rat collagen α1(I) insert was cut out from the original plasmid (pUC18) and inserted into pSafyre. The sections were then hybridized with digoxigenin-labeled collagen α1(I) probe.[34] For immunohistochemistry, TIMP-2 or smooth muscle actin antibodies were used, and the detection was performed with the Histomouse SP kit according to the manufacturer's instructions. To assess cell proliferation, sections were immunostained with PCNA diluted 1:50 with 3% goat serum for 1 hour after 45 minutes incubation with horseradish peroxidase–conjugated goat antibodies to mouse IgG, and peroxidase activity was revealed by using 3, 3-diaminobenzidine (DAB) as chromogen. The results are expressed as a mean of arbitrary units of PCNA index ± SE of 10 sections from 3 animals in each treatment.

Evaluation of Collagen Synthesis. SV40-immortalized rat HSC-T6 line (generously provided by Dr. S.L. Friedman) were incubated with halofuginone in 0.5 mL glutamine-free Dulbecco's modified Eagle medium containing 5% fetal calf serum, ascorbic acid (50 μg/mL), β-aminopropionitrile (50 μg/mL), and 2 μCi of [^{3}H]proline. At the end of incubation, the medium was decanted and incubated with or without collagenase for 18 hours, followed by trichloroacetic acid (TCA) precipitation. The amount of radiolabeled collagen was estimated as the difference between total proline [^{3}H]-containing proteins and those left after collagenase digestion.[26,28] Northern blots analysis was performed as previously described.[28]

RESULTS

Prevention of TAA-Induced Liver Fibrosis by Halofuginone. Rats were treated with halofuginone alone (control group, n = 4), with TAA (n = 6), or with a combination of the two (n = 6). No changes in body weight were observed in any of the rats regardless of the treatment. Spleen weight increased by 11% in the TAA-treated rats compared with the control rats (1135 ± 250 mg compared with 1016 ± 152 mg). No increase in spleen weight was observed in rats that received a combination of TAA and halofuginone (960 ± 65 mg). All rats were sacrificed 12 weeks after initiation of TAA treatment, and the livers were assayed for hydroxyproline content by an amino acid analyzer (Fig. 1). TAA caused a 4-fold increase in liver hydroxyproline content compared with the control group. Halofuginone attenuated the TAA-induced increase in liver hydroxyproline levels and only a 2-fold increase in the hydroxyproline levels was observed (Fig. 1). These results were confirmed by histologic examination of the liver section. The

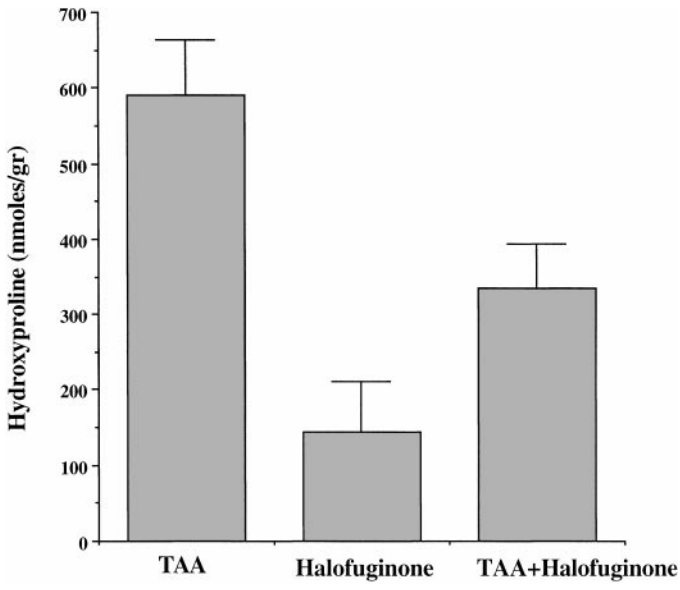

FIG. 1. Hydroxyproline analysis of liver samples. Liver samples were taken from rats treated with TAA (200 mg/kg twice weekly, n = 6), rats treated with halofuginone (5 ppm in diet, n = 4), or rats treated with a combination of the two (n = 6). After 12 weeks, the hydroxyproline levels were measured by amino acid analysis. The results are the mean ± SE of hydroxyproline content in nanomoles per gram of liver tissue.

liver sections of the control rats were devoid of ECM in general (hematoxylin-eosin staining) and of collagen in particular (sirius red staining). When smooth muscle actin antibodies were used, only endothelial cells surrounding blood vessels, but not stellate cells, were detected, suggesting that the latter were in their quiescent state. No cells expressing the collagen α1(I) gene or synthesizing TIMP-2 were detected by *in situ* hybridization or immunohistochemistry, respectively (Fig. 2). When treated with TAA the livers exhibited a marked increase in ECM content and displayed bundles of collagen surrounding the lobules that resulted in large fibrous septa and distorted tissue architecture. These septa were populated by smooth muscle actin–positive cells expressing high levels of the collagen α1(I) gene and containing high levels of TIMP-2, all of which are characteristic of advanced fibrosis. Halofuginone given orally prevented the activation of most of the stellate cells and only traces of smooth muscle–positive cells were detected. The remaining stellate cells expressed low levels of collagen α1(I) gene, which resulted in low levels of collagen. The level of TIMP-2 was almost the same as in the control livers.

Treatment of TAA-Induced Liver Fibrosis by Halofuginone. The possibility that halofuginone may reverse advanced liver fibrosis in rats was evaluated. All rats (n = 22) were treated with TAA and fed a control diet. After 12 weeks, the rats were divided into 3 groups, of which the first (n = 6) were sacrificed on the same day to evaluate the severity of the fibrosis. The remaining 2 groups were fed either the control diet or a diet containing 5 ppm of halofuginone for an additional 8 weeks. At the end of the experiment, liver samples were taken for hydroxyproline analysis (Fig. 3) and for histologic evaluation (Fig. 4). High levels of hydroxyproline were detected in the livers of rats treated with TAA, and these remained unchanged in rats fed for an additional 8 weeks with the control diet. A marked reduction in the hydroxyproline level was observed in the livers of the rats fed halofuginone (Fig. 3),

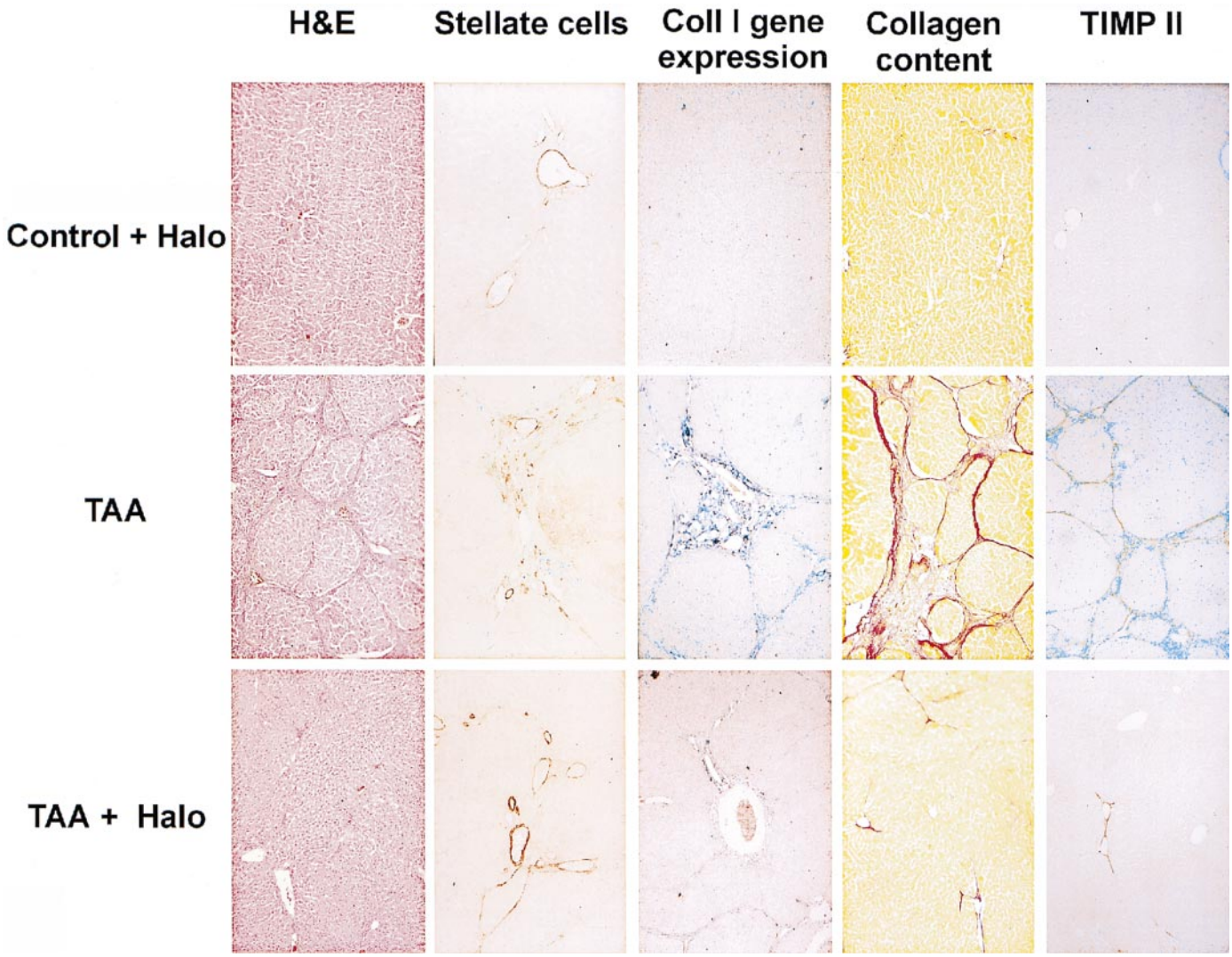

FIG. 2. Histologic analysis of liver sections. Liver samples were taken from rats treated with TAA (200 mg/kg twice weekly), rats treated with halofuginone (5 ppm in diet), or rats treated with a combination of the two. The sections were stained with hematoxylin-eosin and with sirius red for ECM and collagen, respectively. Activated HSC and TIMP-2 were detected by immunohistochemistry with monoclonal antibodies for a smooth muscle actin and with polyclonal antibodies for TIMP-2. Collagen α1(I) gene expression was evaluated by *in situ* hybridization. Note the increase in the smooth muscle actin-positive stellate cells that express the collagen α1(I) gene and synthesize collagen and TIMP-2 after TAA treatment compared with the control. When treated with halofuginone very few activated HSC were observed and they expressed low levels of the collagen α1(I) gene and synthesized traces of collagen and TIMP-2.

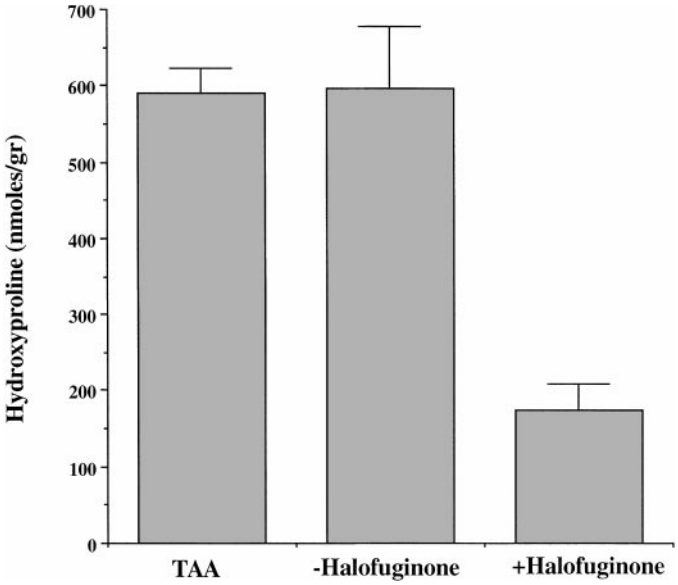

FIG. 3. Hydroxyproline analysis of liver samples. Liver samples were taken from rats treated with TAA 200 mg/kg twice weekly for 12 weeks (n = 6), rats treated with TAA 200 mg/kg twice weekly for 12 weeks and then fed control diet for additional 8 weeks (n = 9), and rats treated with TAA 200 mg/kg twice weekly for 12 weeks and fed halofuginone (5 ppm in diet) for an additional 8 weeks (n = 9). At the end of the experiments the hydroxyproline levels were measured by amino acid analysis. The results are the mean ± SE of hydroxyproline content in nanomoles per gram of liver tissue.

which reached control levels (Fig. 1). These results were confirmed by the histologic examination (Fig. 4). Severe fibrosis was shown after 12 weeks of TAA treatment because of the induction of stellate cells that express high levels of the collagen α1(I) gene, resulting in high levels of collagen deposition. The activated stellate cells synthesize high levels of TIMP-2. The severity of the liver fibrosis was unaltered after an additional 8 weeks of feeding the control diet and no spontaneous resolution of the fibrosis was observed. In contrast, almost complete reversal of the fibrotic condition was observed in rats fed a halofuginone-containing diet (Fig. 4). No smooth muscle actin-positive cells were observed, and cells expressing the collagen α1(I) gene were observed only surrounding central veins. Almost no collagen or TIMP-2 was detected in the liver section.

Effect of Halofuginone on Cell Proliferation. Very few PCNA-positive cells were observed in the pericentral areas of the untreated liver (Fig. 5A). TAA treatment caused a 6-fold increase in the number of PCNA-positive cells (Fig. 5B, 483 ± 50 to 2,920 ± 200), which are probably monocytes and Kupffer cells.[41] Halofuginone prevented 50% of the TAA-dependent increase in the number of PCNA-positive cells (Fig. 5C, 1039 ± 100). Further increase of the number of the PCNA-positive cells was observed 8 weeks after termination of the TAA treatment (Fig. 5D and E 1,889 ± 175 to 2,825 ± 169), which was not observed in the halofuginone-treated rats (Fig. 5F; 1,002 ± 124). Moreover, in the halofuginone-treated rats, the number of the PCNA-positive cells was lower than that observed after 12 weeks of TAA treatment.

Effect of Halofuginone on Collagen Synthesis in HSC **In Vitro.** Incubation of HSC-T6 cells with halofuginone for 24 hours caused a dose-dependent inhibition in collagen synthesis (Fig. 6A). The reduction in collagen synthesis was due to the inhibition of the expression of collagen α1(I) gene (Fig. 6B). More than an hour of treatment was required for any significant inhibition of the collagen α1(I) gene expression.

DISCUSSION

Hepatic fibrosis/cirrhosis is characterized by excessive production of ECM proteins, of which collagen type I is the major one. The collagen is synthesized primarily by the HSC that are activated in the fibrotic liver and that exhibit a myofibroblast-like phenotype. In addition, activated stellate cells synthesize TIMPs (Figs. 2 and 4), which inhibit collagenase activity, thus causing further increase in collagen deposition.[5,42] Thus, pharmacologic intervention in liver fibrosis should be targeted to inhibition of stellate cell activation, inhibition of ECM synthesis, or stimulation of matrix protein degradation. Current therapies for arresting or reversing cirrhosis are largely ineffective and are not targeted to specific elements or steps in the fibrogenic cascade.[24] For example, glucocorticosteroids, which have been shown to inhibit collagen synthesis in culture[43] and in animal models,[44] rarely suppressed fibrogenesis or prevented progression to cirrhosis. In addition, long-term administration of glucocorticoids may cause serious adverse side effects that prevent their use as a general treatment of liver fibrosis. D-penicillamine, which blocks intra- and interchain crosslinking in the newly formed collagen molecules, was found to be ineffective for preventing the progression of hepatic fibrosis and was associated with a high incidence of serious side effects.[24] Halofuginone was found to be an inhibitor of collagen α1(I) gene expression in various animal models in which excessive collagen synthesis is the hallmark of the disease.[25] Clinical safety is a major concern when using an inhibitor of collagen synthesis. We showed that the effect of halofuginone on collagen synthesis was transient, and on halofuginone removal, collagen gene expression *in vitro*[28] and in a cGvHD patient *in vivo*[35] returned to control levels. The reduction in the collagen α1(I) gene expression in rats,[32] rabbits,[27] and in chickens raised on halofuginone-containing diets for 6 weeks from hatching[45] was achieved at concentrations at which no side effects were apparent. Moreover, in all our experiments involving surgery, such as during abdominal or gynecologic adhesion formations[32,33] no abnormal wound healing was observed in the halofuginone-treated animals. Using the wound-tumor model in which C6 glioma spheroids were implanted in surgical incisions, acceleration in wound repair was observed in the halofuginone-treated animals.[46] All of these observations suggest that halofuginone may become a safe therapy for various fibrotic disorders.

In liver, halofuginone was found to prevent fibrosis induced by dimethylnitrosamine[34] or TAA (Figs. 1 and 2). In addition to the known effects on collagen α1(I) gene expression and tissue collagen content, halofuginone treatment caused reduction in the number of activated stellate cells and in TIMP-2 synthesis (Fig. 2). Cell-matrix interactions play an important role in stellate cell activation; collagen type I promotes the entry of stellate cells into S-phase,[47] and loss of normal matrix and its replacement by collagen type I promote stellate cell activation.[48] The three-dimensional structure of the ECM components has been found to regulate the morphology, proliferation, and function of the hepatic stellate cells via integrins,[49] which are known collagen receptors. Furthermore, the function of hepatocytes that are involved in stellate cell activation and recruitment[50] may be perturbed because of an altered cell matrix interaction.[51] Thus, the re-

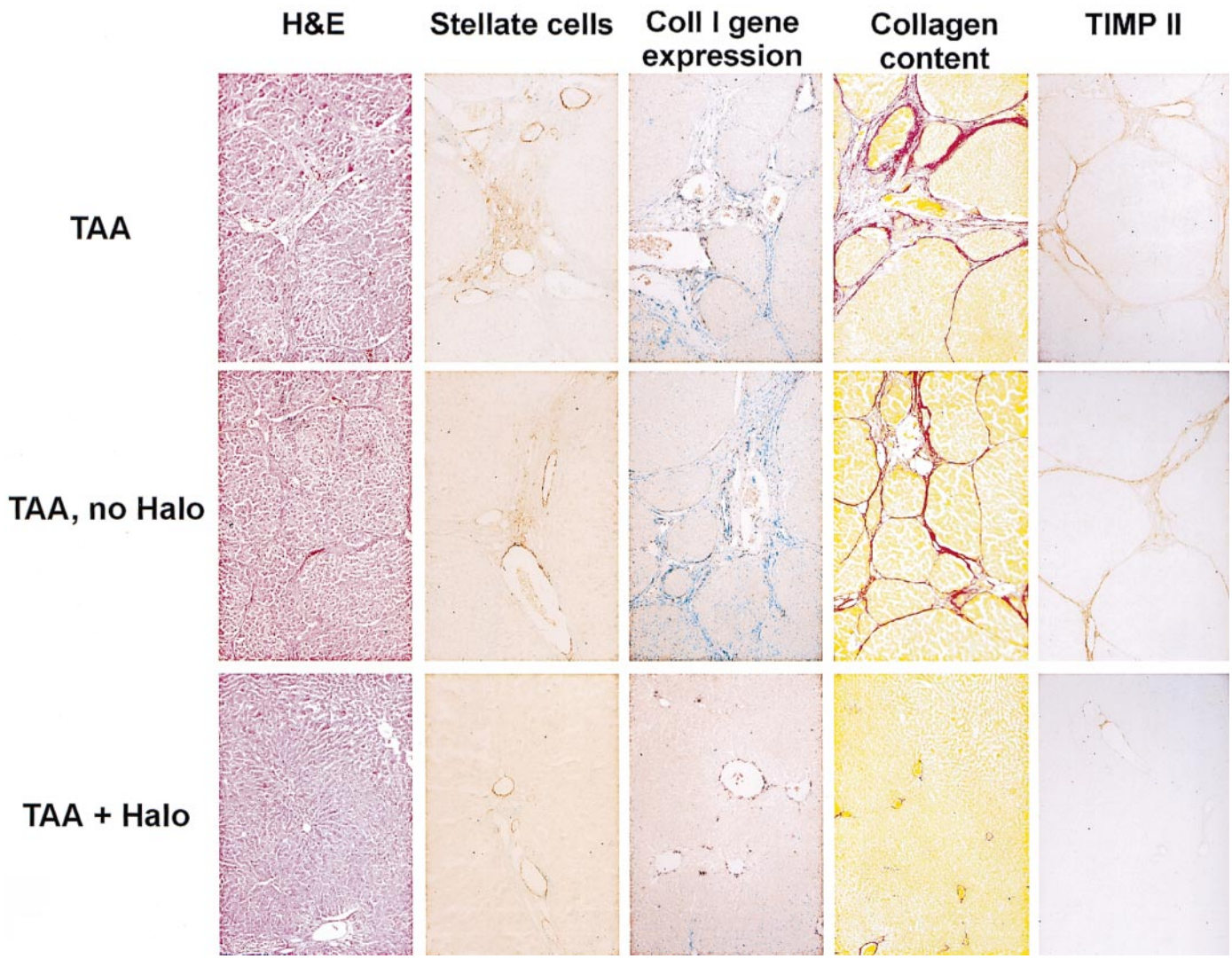

FIG. 4. Histologic analysis of liver sections. Liver samples were taken from rats treated with TAA 200 mg/kg twice weekly for 12 weeks, rats treated with TAA 200 mg/kg twice weekly for 12 weeks and then fed control diet for an additional 8 weeks, and rats treated with TAA 200 mg/kg twice weekly for 12 weeks and fed halofuginone (5 ppm in diet) for an additional 8 weeks. The sections were stained with hematoxylin-eosin and with sirius red for ECM and collagen, respectively. Stellate cells and TIMP-2 were detected by immunohistochemistry, with monoclonal antibodies for a smooth muscle actin and with polyclonal antibodies for TIMP-2. Collagen α1(I) gene expression was evaluated by *in situ* hybridization. Note the high levels of smooth muscle actin-positive stellate cells that express the collagen α1(I) gene and synthesize collagen and TIMP-2 after TAA treatment. Almost complete resolution of the fibrotic lesion was observed after the additional 8 weeks on the halofuginone-containing diet. No spontaneous resolution of the fibrotic lesion was observed.

duction in stellate cell activation may be secondary to the reduction in liver collagen content, which results, on one hand, from the inhibition of the collagen α1(I) gene expression (Fig. 6) and, on the other hand, from the increase in collagen degradation caused by reduced TIMP-2 synthesis. Different fibrogenic cell populations may be involved in fibrosis induced by different agents. For example, an increase in Kupffer cell proliferation was observed in CCl_4-induced fibrosis.[41] It is interesting to note that pentoxifylline, another antifibrotic agent, which has been found to affect collagen content in liver by increased collagen degradation rather than by reduced collagen synthesis,[52] also causes reduction in fibrogenic cell proliferation[41] as does halofuginone (Fig. 5). Using a double staining technique on skin biopsy specimens of the Tsk+ mice—immunostaining with PCNA antibodies and *in situ* hybridization with the collagen α1(I) probe—we showed that halofuginone inhibited cell proliferation only in a subset of fibroblasts that are collagen-producing cells (Pines et al. submitted). These results again suggest that the decrease in collagen α1(I) gene expression by halofuginone is the primary event.

It has been suggested that the inhibition of the collagen α1(I) gene expression by halofuginone requires the synthesis of a new protein(s), probably transcription factors.[28] The fact that more than an hour is required to show inhibition in the collagen α1(I) gene expression (Fig. 6) also suggests some early events. In stellate cells, the proximal promoter (−400 base pairs) of collagen α1(I) contains most of the regulatory regions such as nuclear factor-1 and Sp1 required for accurate expression of the HSC collagen gene at baseline and after injury.[53,54] The DNA fragment flanking the 5′ end of TIMP-2 has several features of regulatory elements shared by housekeeping genes including collagen α1(I), such as Sp1, AP-1 and AP-2.[55] It will be of interest to find out if these two genes are

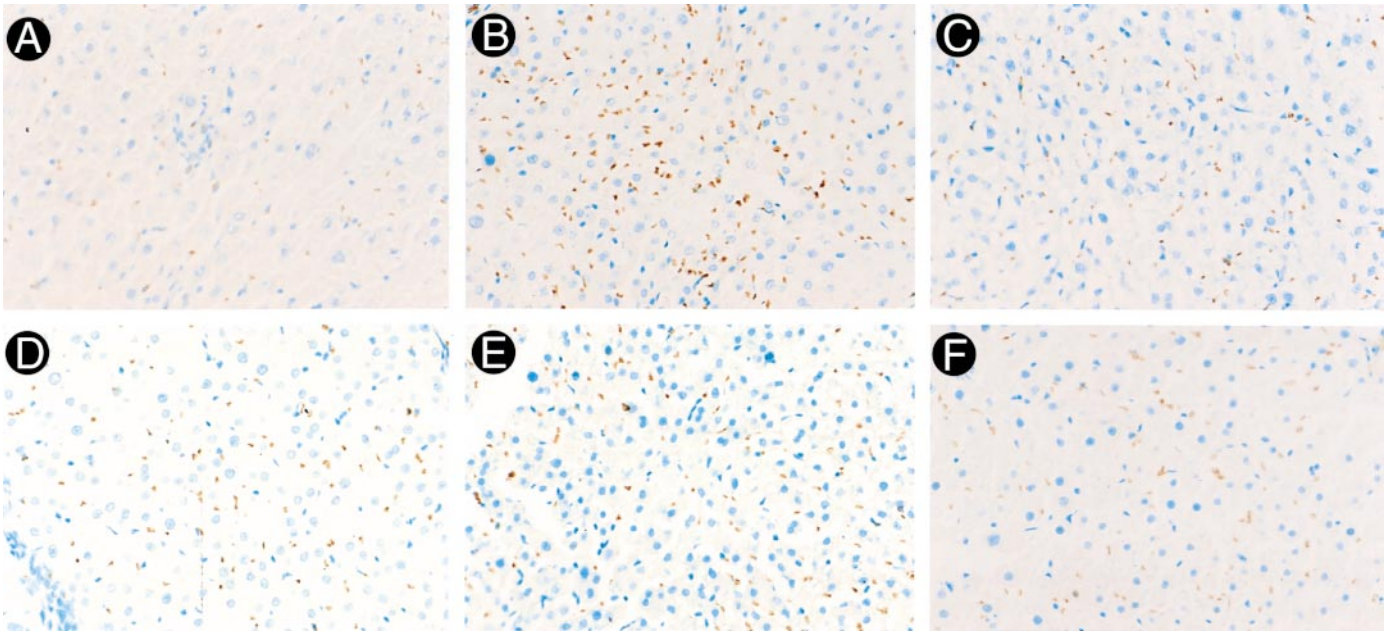

FIG. 5. Immunostaining for PCNA-positive cells. Liver sections were stained with PCNA and the number of positive cells were evaluated by image analysis. *Upper panel*, halofuginone was administered simultaneously with TAA. (A) Control; (B) TAA; (C) TAA + halofuginone. *Lower panel*, halofuginone was administered after fibrosis was established. (D) TAA for 12 weeks; (E) TAA for 12 weeks and an additional 8 weeks normal diet; (F) TAA for 12 weeks and an additional 8 weeks with halofuginone containing diet.

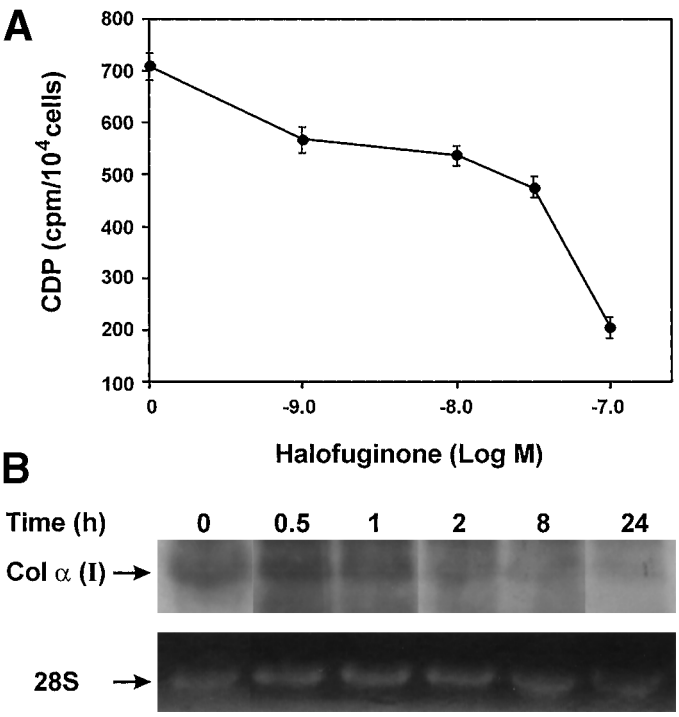

FIG. 6. Effect of halofuginone on collagen synthesis and collagen α1(I) gene expression in HSC-T6. (A) Cells were incubated for 24 hours with 2 μCi of [^{3}H]proline and various concentrations of halofuginone. At the end of the incubation the collagenase digestible proteins (CDP) were evaluated. (B) Northern blot analysis using the rat collagen α1(I) probe. Cells were incubated with halofuginone (10×10^{-8} mol/L) for various intervals. The 28S RNA was used as a control for the quantity of the RNA loaded in each lane.

regulated in concert and whether the putative transcription factor would simultaneously inhibit the collagen α1(I) and TIMP-2 genes. Cell types in various tissues other than stellate cells clearly need additional regulatory regions of the upstream promoter sequence and/or the first intron, which emphasizes the complex cell-specific transcription factors that are likely to exist. The inhibition of collagen synthesis by halofuginone is not cell-type specific, which suggests a more general mechanism of inhibition. Thus, in addition to HSC (Fig. 6), halofuginone may inhibit collagen synthesis and deposition by other cell types that contribute to the fibrotic lesion.[56]

Advanced fibrosis and cirrhosis are generally considered to be irreversible conditions, even after removal of the injurious agent. However, there is evidence that recovery, with remodeling of excess ECM proteins is possible.[57-59] In TAA-induced liver fibrosis, no spontaneous resolution was observed. High numbers of HSC, high levels of collagen synthesis (Fig. 4), and high numbers of PCNA-positive cells (Fig. 5) were observed 8 weeks after cessation of the insult. Halofuginone given orally caused almost complete resolution of existing fibrosis (Figs. 3 and 4). These results are in agreement with our previous observations that prolonged halofuginone treatment may resolve existing fibrosis in the TSK mice[29] and in a cGvHD patient.[35] Halofuginone treatment probably perturbs the balance between synthesis and degradation of collagen. Halofuginone inhibits the collagen α1(I) gene expression (Fig. 6) and, by inhibiting the synthesis of the metalloproteinase inhibitors, it probably augmented collagenase activity, causing collagen degradation and resolution of the fibrotic lesion.

In summary, up-regulation of collagen synthesis appears to have a critical role in the pathophysiology of liver fibrosis. By affecting various checkpoints in the development of hepatic fibrosis, halofuginone shows promise of becoming a novel antifibrotic agent for prevention and treatment of liver fibrosis.

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