Advances in the pathogenesis of liver cirrhosis

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Cirrhosis of the liver is a common problem in clinical practice worlwide and is among the ten most common causes of death in adults in developed countries (1-4). This disorder is characterized by widespread fibrosis and the formation of regenerative nodules in the liver. Although the causes of cirrhosis are many (Table 1), the end result is the same and its pathogenesis is not known (1-4).

In physiological conditions liver cells produce the right amount of connective tissue components in the correct places involving highly coordinated proccesses which maintain normal phenotypic expression (2, 4). In liver cirrhosis homeostasis is altered and excess deposition of extracellular matrix in abnormal locations is accompained by distortion of hepatic structure, hemodynamic alterations and impairment of function (1-4).

In the last years viral chronic hepatitis and cirrhosis has been the focus of attention of many clinicians and biologists and new data are now available on the etiopathogenesis of these disorders (5).

Chronic viral infections represent an important group of cases of human chronic liver disease (1-5). While in most of these patients the hepatic disease is mild, sometimes a progressive process leading to liver cirrhosis is observed (5,6). Progression in HBV related liver disease inflammation and fibrosis seems to depend on a combination of active viral replication in the liver and an abnormal immunological background of the patient (2-8).

On the other hand, it has recently been shown that hepatitis C virus (HCV) is the etiological agent of most cases of transfusion associated and sporadic non-A non-B (NANB) hepatitis (9-13) and this agent has also been implicated in many cases of cryptogenic cirrhosis (12, 14). Chronic elevation of aminotransferase activities follows in aproximately 50% of cases of acute NANB hepatitis and 20% of these patients have morphologic evidence of cirrhosis when first biopsied (15). Insidious progression of chronic NANB hepatitis to cirrhosis is emerging as a consistent observation even though patients may be asymptomatic and have only marginal elevations of aminotransferase activities (15).

Liver fibrosis

Although fibrosis is a common component of distinct forms of chronic hepatic disease, it seems to occur by several different mechanisms (16, 17). While ethanol and possibly iron have direct fibrogenic effects in the liver (18, 19), viral infections indirectly induce this process (16, 17). HBV and HCV have antigenic structures which elicit an immune response from the host (2-6, 8, 16, 17, 20). In viral chronic liver disease, the stimulus for fibrogenesis seems to arise from the hepatic inflammatory infiltrate and involves local production of cytokines and other mediators (16, 17).

Little information is available on the pathobiology and natural history of fibroplasia in human cirrhosis (21). Normal human liver contains approximately equal quantities of type I and III collagens and a lesser amount of type IV, V and VI fibrils (16, 17, 22, 23). In addition, basement membrane matrix contains type IV collagen and glycosilated proteins, such as fibronectin and laminin (16, 17, 23).

Table 1

AETIOLOGY OF CIRRHOSIS
Alcohol
Viral hepatitis:
type B
type B and D
type C
Metabolic disorders:
Alfa1-antitrypsin deficiency
Haemocromatosis
Wilson's disease
Cystic fibrosis
Porphyria
Abetalipoproteinemia
Byler's disease
Galactosaemia
Hereditary fructose intolerance
Venous outflow obstruction:
Veno-occlusive disease
Budd-Chiari syndrome
Cardiac failure
Drugs and toxins
Intestinal bypass for obesity
Biliary disease:
Extrahepatic biliary obstruction
Intrahepatic biliary obstruction:
Primary biliary cirrhosis
Primary sclerosing colangitis
Autoimmune chronic hepatitis

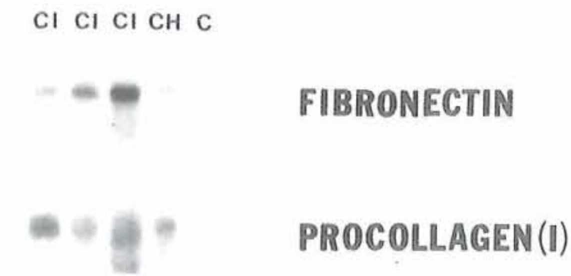
In hepatic fibrotic diseases there is an increase in the liver content of fibrillar collagens I and III (16, 17, 23) and immunohistochemical studies suggest that the space of Disse in these disorders also has augmented amounts of fibronectin and laminin (16, 17, 23, 24).

These changes in liver matrix in hepatic cirrhosis represent the accumulation of abnormal amounts of normal proteins, but it is unknown whether these increments are due to increased synthesis, decreased degradation or a combination of both processes.

Fibrogenesis markers

Studies with new drugs having the potential to specifically inhibit hepatic collagen synthesis in the liver demand for serum tests to monitor the effectiveness of such therapies (25). Now we will review some published studies (26, 27) and give some new data on liver and serum fibrogenesis markers in patients with chronic viral liver disease.

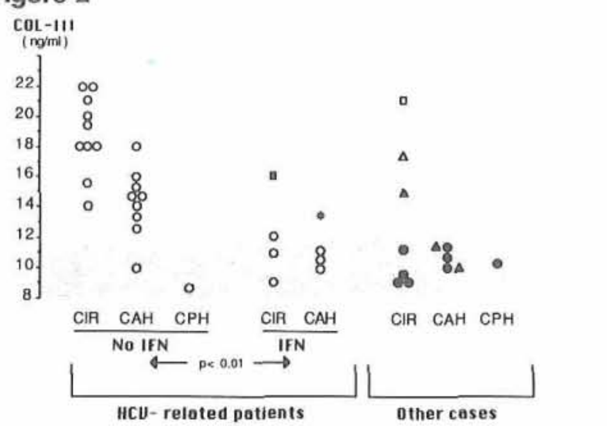
Figure 1



Representative Northern blots for procollagen-α_1 (I) and fibronectin using RNA extracted from controls (C) and patients with chronic hepatitis (CH) and cirrhosis (CI). The liver expression of both mRNAs share a similar pathern.

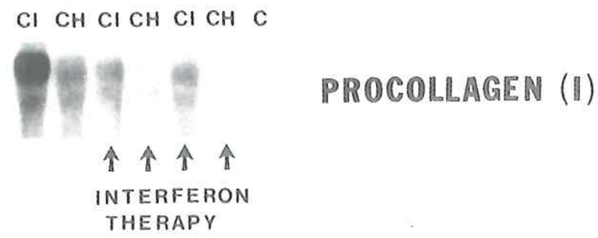
We studied the steady-state levels of messenger RNAs (mRNA) for procollagen-α_1 (I) and fibronectin in liver samples of patients with viral chronic hepatitis and cirrhosis along with the serum concentration of procollagen type III aminoterminal peptide (PIIIP), a structure cleaved off the procollagen protein in the synthesis of a collagen type III fibril (16). In some patients with HCV chronic infection these parameters were

Figure 2



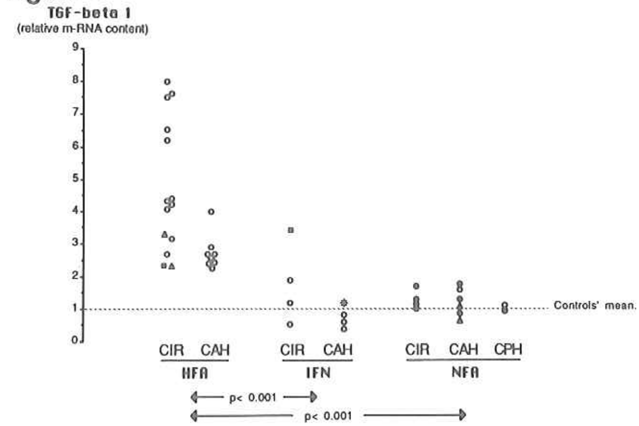
Serum levels of aminoterminal peptide of procollagen type III in patients with hepatitis B virus (HBV) chronic infection and hepatitis C virus (HCV) chronic liver disease [some of them after twelve months of treatment with lymphoblastoid α-interferon (IFN)] (CIR = cirrhosis; CAH = chronic active hepatitis; CPH = chronic persistent hepatitis; o = infection by HCV; ■ = case with infection by HCV who did not respond to IFN; * = case with infection by HCV and partial response to IFN; ▲ =infection by HBV and positive viral DNA in serum; • = infection by HBV and negative viral DNA in serum).

Figure 3



Representative Northern blots for procollagen-α_1 (I) using RNA extracted from controls (C) and patients with hepatitis C virus chronic hepatitis (CH) and cirrhosis (CI). In patients treated with lymphoblastoid α-interferon the procollagen I message expression is disminished.

Figure 4



Relative steady-state levels of messenger RNA for transforming growth factor beta-1 (TGF-beta1) in patients with high fibrogenic activity [HFA; serum PIIIP above the mean+2 standard deviations of controls (11.8 nanograms/ milliliter)], patients with HCV chronic disease after 12 months of treatment with α-IFN and patients with normal fibrogenic activity (NFA) non treated with IFN (CIR = cirrhosis; CAH = chronic active hepatitis; CPH = chronic persistent hepatitis; o = infection by HCV; ■ = case with infection by HCV who did not respond to IFN; * = case with infection by HCV and partial response to IFN; ▲ = infection by hepatitis B virus and positive viral DNA in serum; . • = infection by hepatitis B virus and negative viral DNA in serum; = primary biliary cirrhosis; Δ = case associated to ulcerative colitis).

studied after one year of treatment with lymphoblastoid α-interferon (α-INF). These cases were included in a randomised clinical trial (28).

In 37 patients with hypertransaminasemia and clinical data suggesting chronic liver disease, or previously diagnosed of viral chronic liver disease, and in 8 cases with normal liver functional test who underwent surgery for colecystectomy a liver functional tests who underwent surgery for colecystectomy a liver biopsy was taken with a Trucut sheathead. Thirty-five patients had chronic viral inflammatory liver disease (19 cirrhosis and 16 chronic hepatitis; 9 chronic infection with HBV and 26 with HCV) and two had hepatic steatosis. The eight surgical cases showed normal histology in the liver and were included as controls in the RNA studies along with the two fatty liver samples.

Eight out of the 26 patients with chronic infection by HCV were studied after one year treatment with low doses of α-IFN as published previously (28). Six of the cases demonstrated a full response (stable normalization of aminotransferase concentrations during treatment), one a transient response (normalization of aminotransferase levels during treatment) and the other one was a non-responder (no change in aminotransferase levels during therapy). RNA extraction from liver samples, Northern blotting, viral markers, PIIIP levels and statistics were carried out as described (26).

Most patients with cirrhosis and some cases with chronic active hepatitis had an increased liver content of mRNA for procollagen-α_1 (I) (Fig. 1). The higher levels of expression were found in patients with active cirrhosis related to chronic HCV infection. In this group, steady-state levels of procollagen I mRNA ranged from 3 to 8.5 fold above the amounts found in the control group. Procollagen I message expression correlated directly with the biochemical activity (ALT level) of the liver disease ($r=0.62$; $p<0.001$). Thus, patients with inactive cirrhosis or persistent chronic hepatitis had a normal or practically normal mRNA liver content for these molecules.

Increments in the expression of procollagen I mRNA correlated with changes in the liver content of fibronectin message ($r=0.79$; $p<0.001$; Fig. 1). However, increments in the expression of the latter were less intense (1-2.5 fold above controls levels).

Serum PIIIP were also elevated in most patients with liver cirrhosis and some cases with chronic active hepatitis (Fig. 2). There was a direct correlation between the serum concentration of this peptide and the liver content of mRNAs for procollagen I ($r=0.84$; $p<0.001$) and fibronectin ($r=0.77$; $p<0.001$). Serum PIIIP levels also correlated with the ALT concentration in serum (r=0.60; p<0.001). These data show that PIIIP is a good serum indicator of hepatic fibrogenesis and agree with other studies (16, 22, 23, 25).

α-IFN therapy induced a decrease in the fibrogenic activity of the disease in patients with HCV chronic infection who responded to the treatment. Steady-state levels of liver procollagen I message were normalized in responders to interferon (Fig. 3) along with the serum levels of procollagen III peptide (Fig. 2). In the case who showed a transient response to interferon the procollagen I mRNA expression was also normal but it remained high in the patient who did not respond to the therapy. Procollagen III peptide levels at the end of interferon treatment remained elevated in both cases (Fig. 2).

Mecanisms of liver fibrosis: increased fibrogenesis

These data show a substantial increase in mRNA levels for procollagen type I in the liver of patients with active viral chronic liver disease. Similar results have also been reported recently by Annoni et al. (29). In our study, as in other series (16, 22, 23, 25), high serum levels of PIIIP were found in these patients, indicating an augmented rate also in the production of these molecules. Furthermore, in those patients with high levels of collagen synthesis parameters, a correlative increase in steady-state liver content of mRNA for fibronectin was also detected. Taken together, these results suggest that stimulation of extracellular matrix proteins production is likely to be a significant factor in the genesis of hepatic fibrosis in human viral cirrhosis.

A correlation was found between the increments in fibrogenesis markers and the biochemical activity of the disease. Thus, patients with inactive cirrhosis or chronic hepatitis had normal fibrogenesis parameters sugesting that in these cases the fibrotic process is not progressing.

Clinicians need to monitor connective tissue deposition in patients with chronic liver disease. In this regard, it is interesting to notice that the serum PIIIP, a test easy to perform and with a known normal interval, correlated closely in our patients with the amount of liver message for both procollagen type I and fibronectin suggesting that this biochemical parameter may be used as arealiable monitoing probe for the fibrogenic activity of viral chronic hepatitis and cirrhosis.

Alfa-interferon and liver fibrosis

A few years ago, several preliminary reports suggested that both interferon α and ß might be useful in the treatment of patients with chronic HCV infection (30-34) and some recent randomized clinical trials have demonstrated beneficial effects of interferon α in this disease (35-42).

It seems that three doses weekly of at least three millions units of α-interferon have to be continued for more than six months to obtain a response in most of the patients. In the cases who respond to α-interferon normalization in serum aminotransferase levels is observed accompained by a disminution in piecemeal hepatocyte necrosis and lobular injury indexes in liver histology.

In addition, in our study treatment with α-IFN for one year induced in patients who responded to the therapy a normalization in liver and serum biochemical parameters of fibrogenesis suggesting that α-IFN abolish the deposition of extracellular matrix proteins in the liver of these patients.

Transforming growth factor-ß and hepatic fibrogenesis

It is assumed that all the liver cells involved in fibrosis may eventually produce both collagens and collagenases (22). The regulating mechanisms of such dualism are probably modulated by growth factors and cytokines such as transforming growth factor (TGF) ß-1, a protein able to increase the synthesis of collagens and other extracellular matrix proteins and to diminish their degradation (43-45).

In cultured cells, TGFß1 stimulates the synthesis of collagens and other extracellular matrix components and decreases their degradation (43-45). In addition, TGFß1 is capable of activating lipocytes (Ito cells) (23, 48), the cell line that is presumably a major site of synthesis of matrix proteins in chronic liver disease (23, 48-50).

A parallel increase in hepatic TGFß1 and procollagens mRNAs has been observed in two experimental models of liver fibrosis using Northern blotting and *in situ* hybridazation (46, 51). In carbon tetrachloride-induced rat liver fibrosis, Nakatzukasa et al. (51) detected increased levels of TGFß1 mRNA in lipocytes, myofibroblasts and fibroblasts. At the early stages of fibrosis TGFß1 mRNA was also detected in inflammatory cells infiltrating the liver.

In other studies (25), we have demostrated increased steady-state levels of mRNA for TGF-ß1 in the liver of patients with viral chronic hepatitis and cirrhosis with high fibrogenesis parameters (liver procollagen I mRNA expression and serum levels of PIIIP) (Fig. 4). On the other hand patients with normal fibrogenic activity had hepatic TGF-ß1 mRNA levels similar to those of the control group. Furthermore, TGF-ß1 message expression correlated closely with the mRNA liver content for procollagen I and with the serum levels of PIIIP.

These data show that TGF-ß1 plays a central role in the pathogenesis of fibrosis in patients with viral chronic liver disease (25).

Interestingly, in those patients with HCV chronic infection who responded to α-interferon therapy, TGF-ß1 mRNA expression became normal (Fig. 4). This fact indicates that the effects of α-interferon on procollagen gene expression in HCV chronic liver disease may be at least partially secondary to changes in TGF-ß1 levels. Recently it has been shown that γ-interferon is able to diminish in a dose-dependent manner the expression of mRNA for procollagen type I and III by liver cells both *in vitro* and *in vivo* (45) suggesting the possibility that also α-interferon might have direct effects on liver fibrogenesis.

Transforming growth factor-α in regeneration of livers with cirrhosis

Hepatic cirrhosis is characterized by, in addition to a diffuse process of fibrosis, the formation of regenerative nodules in the liver (1, 2, 4). Hepatic cell proliferation in cirrhosis is probably important in order to mantain a sufficient number of hepatocytes able to keep normal liver function. In fact, decreased liver volume, reflecting a diminished number of functioning liver cells, is a sign of poor prognosis in hepatic cirrhosis (4).

Both TGF-α and TGF-ß1 have been implicated in experimental liver regeneration (52-55). Altought they share a similar name, they have entirely different structures, messenger RNA sizes, cellular receptors and functional actions (52-55). TGF-α and TGF-ß1 act as positive and negative specific signals respectively in the regulation of liver regeneration after partial hepatectomy in rats. TGF-α is produced in the liver by hepatocytes and TGF-ß1 by nonparenchymal cells, and they have specific effects on hepatic cells both *in vitro* and *in vivo* (52-55). These data suggest that TGF-α might be important in the formation of regenerative nodules in human cirrhosis.

We have investigated TGF-α expression in the liver of patients with viral chronic liver disease (25). To determine whether this protein might be associated with active cell proliferation in the liver we compared the levels of TGF-α mRNA hepatic expression with those of H3 histone gene mRNA, a good marker of DNA synthesis (56-58). H3 histone gene and TGF-α mRNAs were detected in all the patients with regenerative nodules but only in some cases with chronic active hepatitis. TGF-α message liver content correlated closely with that of H3 histone gene suggesting that TGF-α may be a key regulating factor in liver cell proliferation in human cirrhosis (25).

Conclusion

In summary, this review shows that an increased production of extracellular matrix proteins is an important factor in the production of liver fibrosis in viral cirrhosis and that the serum levels of PIIIP is a good marker to monitor fibrogenesis in viral chronic liver disease.

In addition, α-IFN is able to diminish the fibrogenic activity of the disease in those patients with chronic HCV infection who respond to therapy.

Finally, recent data indicate that TGF-β1 plays a central role in the induction of fibrogenesis in chronic hepatitis and cirrhosis and that TGF-α may be important in the regulation of liver cell proliferation in these patients.

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