Hepatic fibrogenesis

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Abstract

Progress achieved in chronic liver disease treatment has also generated increased interest in clarifying the mechanisms underlying the development of hepatic fibrosis. After a liver injury activation of hepatic stellate cells (HSC) and their conversion to contractile, proliferative and fibrogenic cells responsible for the production of major extracellular matrix proteins (MEC) are the current focuses of investigation. However, the prospect of reversing hepatic fibrosis through the discovery of new antifibrotic therapies is even more attractive to researchers. This paper reviews these developments and ends with a view of the current state of human clinical trials.

Keywords

Cirrhosis, stellate cells, fibrosis, matrix.

In recent years there has been great interest in the possibility of achieving liver fibrosis regression, in part due to results achieved in treatment of chronic hepatitis C (1). However, there is now a controversy about the meaning of the terms "reversal" and "regression" in relation to fibrosis/cirrhosis of the liver (1, 2). Reversal of cirrhosis involves complete restoration of the hepatic architecture to its normal state and the disappearance of the cirrhosis. Regression is "improvement" of fibrosis/cirrhosis to a lower grade than that initially found, in some cases to normalcy (1). The ability to stop fibrosis/cirrhosis, or to make it disappear, has been a major focus of research in recent decades challenging the older concepts about the irreversibility of hepatic fibrosis. It was once considered to be a one directional alteration without possibility of return (1, 2). However, nowadays regression of fibrosis and cirrhosis in various chronic liver diseases has been demonstrated, (1-3) suggesting that in hepatitis C, the goal of treatment should be regression of cirrhosis rather than simply sustained viral response.

THE PROCESS OF HEPATIC FIBROGENESIS

Liver fibrosis is considered a curative response, which is intended to limit the tissue damage produced by chronic liver lesion regardless of etiology (1, 4), but when the slight is persistent, the healing process can produce result in the appearance of liver cirrhosis resulting in altered architecture characterized by bands of fibrosis, regenerative parenchymal nodules and vascular distortion (3-5). The composition of liver fibrous scarring is similar for all lesions regardless of the cause of the lesion (hepatitis B or C viruses, drugs, alcohol, autoimmune or metabolic diseases such hemochromatosis, Wilson's disease, etc.) (3-5). Fibrosis occurs in the sites of the largest lesions, usually only after the harmful stimulus has persisted for many months or years (7, 8). Although classically this process was considered to be irreversible (6), clinical and experimental evidence suggests the contrary (4, 7, 8). Evidence to the contrary includes a review of histological samples and treatment records of patients who had had

chronic liver diseases of various etiologies and which were successfully treated, and animal models of fibrosis indicating fibrosis is a bidirectional dynamic process in which recovery and remodeling of scar tissue can occur especially in the initial stages (7, 8). However, the point of no return from which moment on cirrhosis becomes irreversible is still unknown (1-3). For fibrosis to begin elements derived from damaged hepatocytes are required, although the presence of inflammatory cells is not always necessary. This has been demonstrated in hemochromatosis in which there are no inflammatory cells (8, 9).

Histologically the liver is composed of parenchymal cells (hepatocytes) and non-parenchymal cells (2, 3). Hepatocytes represent 80% of liver volume while non-parenchymal cells represent 6.5% of total liver. 40% of the latter are found in the hepatic sinusoids. The hepatic sinusoids have three types of cells: endothelial cells, Kupffer cells and hepatic stellate cells (HSCs) (3). It is estimated that there are 109 HSCs per 1000 hepatocytes in rat livers (10). The HSCs are perisinusoidal cells located in the subendothelial space of the space of Disse. In their quiescent state their primary function is to serve as repositories of retinoids (vitamin A and its metabolites) (8-10). Through the process of activation, aggressive stimuli such as viruses, alcohol, or any xenobiotic, transform these cells into totally different entities that are morphologically similar to myofibroblasts but with additional functions including production of extracellular matrix (ECM) and multiple proinflammatory cytokines (7-9). Activation of HSCs is characterized by myofibroblast proliferation, contractile activity and fibrogenesis. The HSCs, also known as Ito cells, were once called lipocytes or fat-storing cells. They were identified in the 1990s as an important source of collagen in the liver. With that knowledge, hepatic fibrosis began to receive greater attention (8, 13). These cells are in physical contact with hepatocytes and sinusoidal endothelial liver cells with cytoplasmic extensions. It is estimated that 85% of the vitamin A in the liver is in the HSCs (13, 14).

Consensus now exists that activation of HSCs is the centerpiece of liver fibrogenesis. Moreover, in addition to their ability to produce fibrosis these cells also act as antigen presenting cells (APC) and progenitor cells capable of differentiating into endothelial cells and hepatocytes. This highlights their high functionality and leading role in liver regeneration (7). There is also consensus cellular sources of myofibroblasts other than HSCs contribute to liver fibrogenesis. The most studied of these are progenitor cells from the bone marrow, portal fibroblasts and mesenchymal cells from transitional hepatocytes and cholangiocytes (7, 8, 12).

HSC activation mechanisms which lead to their transformation from quiescent cells to myofibroblasts are complex and diverse since the actions of different types of cells influence HSCs (13, 14).

THE ROLES OF DIFFERENT TYPES OF CELLS

HSC activation may be the result of interaction of different types of liver cells including activated macrophages (Kupffer cells), damaged or injured hepatocytes, platelets and endothelial cells. The consequent production and release of different cytokines and oxygen free radicals (OFR) together stimulates and activates HSCs (13, 14). A cascade mechanism has been proposed involving these cells during a pre inflammatory stage, an inflammatory stage and a post-inflammatory stage (7). The influence of these types of cells during fibrogenesis is shown in Figure 1.

KUPFFER CELLS

These cells are macrophages whose main activity is to remove and detoxify exogenous and endogenous agents particularly those from intestinal bacterial endotoxins. These include bacterial lipopolysaccharide (LPS), a strong inducer of inflammation (9). The response of Kupffer cells to a damaging stimulus activates HSCs. Activation induces mitotic activity by the HSCs which has important additional effects. These include phenotypic transformation of HSCs to myofibroblasts; increased synthesis of proteins, elastin and collagen; stimulation of proliferation; and growth factor response to platelet-derived growth (PDGF) (9, 13-15).

MYOFIBROBLASTS

Activated HSCs participate in activation of additional HSCs through autocrine and paracrine mechanisms and through cytokines and growth factors. Growth factors generate differential expression of ECM proteins. The best studied of these are the transforming growth factor-beta (TGF-β1), transforming growth factor alpha (TGF-α) and connective tissue growth factor (CTGF). All three are highly fibrogenic proteins (7).

HEPATOCYTES

Since HSCs and hepatocytes are in close proximity they are permanently in contact, either directly through their cell membranes or through soluble mediators which can activate HSCs (7, 8). The exposure of hepatocytes to cytotoxic agents makes them release mitogenically active substances like PDGF. It may also promote induction of collagen type I, as in the case of acetaldehyde (7). The partial destruction

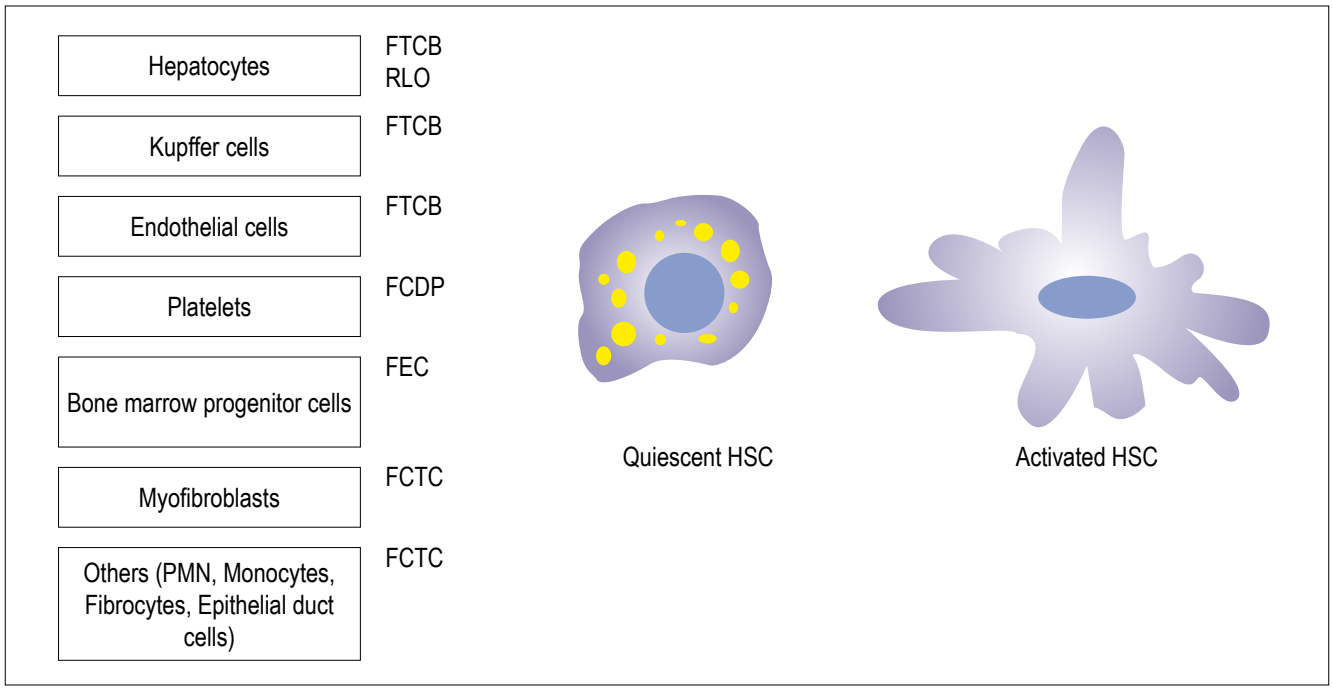

Figure 1. Different cell types involved in the activation of HSCs and mediators. Their Spanish acronyms appear in the figure. FCT B stands for growth factor beta. OFR stands for oxygen free radicals. PDGF stands for growth factor derived from platelets. FEC stands for Colony Stimulating Factor. FCTC stands for connective tissue growth factor.

and apoptosis of hepatocytes are also mechanisms involved in activation of HSCs (13, 14).

PLATELETS

Because of their presence in areas of inflammation and necrosis areas platelets are an important source of pro-inflammatory and pro-fibrogenic cytokines such as TGF and PDGFB which promote growth transformation and ECM synthesis (15).

PROGENITOR CELLS FROM BONE MARROW

Progenitor cells from bone marrow have the potential to differentiate into hepatocytes, cholangiocytes, sinusoidal endothelial cells, Kupffer cells, HSCs or myofibroblasts depending on the microenvironment (7). There is a subpopulation of circulating leukocytes with phenotype CD45 + with hematopoietic origin in CD34 + which are capable of inducing matrix synthesis (7, 9).

PERIPHERAL BLOOD CELLS

Evidence suggests that a subpopulation of monocytes can differentiate into hepatocytes, interleukins or fibrocytes depending on specific stimuli and when stimulated by colony stimulating factors (M-CSF) (7, 9).

EPITHELIAL-MESENCHYMAL TRANSITION OR TRANSFORMATION (EMT)

HSCs come from septum transversum mesenchyme, endoderm or Glisson's capsule (mesothelial capsule of the liver). However, observation of a reaction against fibrotic liver damage has led to the proposal of an additional mechanism for the generation of fibroblasts via the transdifferentiation of epithelial cells into fibroblasts (4, 8). After EMT hepatocytes can express type I collagen synthesis, or cholangiocytes can then express ESP1 and vimentin as early fibroblast markers. Thus, the direct consequences are ductopenia and portal fibrosis resulting from an augmented pool of fibroblasts. This seems to be one of the most important pathogenic mechanisms of chronic cholestatic diseases such as primary biliary cirrhosis (7, 9, 19).

Molecular inducers of EMT include TGF-β, epidermoid growth factor (EGF), insulin-like growth factor (IGF), and basic fibroblast growth factor (FGF-2). All of these promote the programming of epithelial cells to become mesenchymal cells phenotypically and genotypically. However, the prototype of the most powerful inducers of

EMT is TGF-β which induces EMT by either activating phosphorylation of Smad 2/3, or by inhibition of Smad 4 silencing through RNA interference (6, 8).

The activity of TGF-β is also expressed through the production of CTGF, a strong inducer of ECM and proliferation of fibroblasts. It is currently used as a marker for liver fibrosis (7). Only hepatocytes resistant to apoptosis induced by the same cytokine are induced to EMT (4, 5). The inverse of epithelial mesenchymal transition has been observed in the bone and in blood vessels, but has not yet been established in the liver (7).

STAGES OF FIBROGENESIS

Friedman describes the process of fibrogenesis in three phases: initiation, perpetuation and resolution (8).

Initiation (7-9)

Initiation refers to primary phenotypical changes of HSCs which provides greater responsiveness to growth factors and ultimately provides increased synthesis of ECM molecules. The initial changes are paracrine signals through different molecules and cytokines originating in hepatocytes, Kupffer cells, leukocytes, and sinusoidal endothelial cells as described earlier.

Perpetuation

Perpetuation involves at least seven changes in cell behavior: proliferation, chemotaxis, loss of retinoids, release of cytokines, contractility, fibrogenesis and extracellular matrix degradation.

Proliferation

Increased secretion of growth factors and increased secretion of cytokines with mitogenic power result in proliferation. For HSCs, PDGF is the most mitogenic powerfully cytokine that has been characterized (11, 15). Receptor induction occurs very early in activation. Other factors which act mitogenically on HSCs include vascular endothelial growth factor, thrombin and its receptors, and TGF-α(8).

Chemotaxis

The migration of HSCs to sites with hepatocellular damage is due to the chemo-attractant activity of substances such as PDGF, MCP-1 and CXCR3 (12).

Loss of retinoids

Activation of HSCs, is accompanied by loss of the perinuclear retinoid accumulation (7, 8). 80% of total body vitamin A is deposited in the HSCs as retinol esters and especially as free retinol in lipid droplets which are recognized as fat deposits. Although tLa composición de las gotas es afectada por la dieta y también contienen triglicéridos, fosfolípidos, colesterol y ácidos grasos libres.he composition of the droplets is affected by diet, they contain triglycerides, phospholipids, cholesterol and free fatty acids. Su comportamiento con respecto a estos rasgos adipogénicos tiene múltiples evidencias y paralelos con los adipocitos, pues han sido caraterizados en ella los efectos de la leptina, adiponectina y el PPAR (gamma) como también los efectos anti-adipogénicos del FNT alfa y el Wnt.There is a large quantity of evidence regarding their behavior with respect to these adiposity traits which is like that of adipocytes. The effects of leptin, adiponectin and peroxisome proliferator-activated receptors (PPARs)within them have been well characterized, as have the anti-adipogenic effects of tumor necrosis factor-alpha (TNF alpha) and Wnt (8,16-18). El cambio morfológico de la pérdida de los retinoides es una condición necesaria para la modificación del citoesqueleto de las CEHs activadas y los eventos posteriores, pero aún no son claros los mecanismos intracelulares que lo permiten y los efectos de los retinoides sobre las CEHs y la fibrogénesis, son contradictorios. (12, 14,17). (8, 16-18). Although morphological changes resulting from the loss of retinoids are necessary for modification of the cytoskeleton of the activated HSCs and for subsequent events, the intracellular mechanisms which permit these changes are not yet clearly understood while the effects of retinoids on HSCs and fibrogenesis are contradictory (12, 14, 17).

Fibrogenesis (7, 8, 13)

HSCs generate fibrosis not only by increasing the number of cells, but also by increased production of extracellular matrix. Collagen type I is the prototype constituent of the matrix of fibrotic livers. Its expression is regulated by transcriptional and post-transcriptional mechanisms. The most powerful stimulus for increased production of collagen type I is TGF-β1. TGF-β1, derived from paracrine and autocrine sources, remains as the classic fibrogenic cytokine. Another way TGF-β1 stimulates synthesis of collagen is through hydrogen peroxide and a mechanism dependent on C/EBPβ (7, 8, 13). Another potent fibrogenic signal for HSC mentioned above is connective tissue growth factor CTGF/CCN2. Although it is regulated by conditions such as hyperglycemia and hyperinsulinism, it has been shown that its regulation is also dependent on TGF-β1 hepatocyte (19).

As for neurohumoral fibrogenic effects, cannabinoids have recently emerged as mediators of hepatic steatosis, activation of HSC, and hepatic hemodynamic alterations in advanced disease. It has been established that CB1 fibrogenic receptors and CB2 antifibrotic receptors exert opposite

effects. These have recently become the focus of study as promising therapeutic strategies (20).

Fibrosis is characterized by several steps that lead to increased extracellular matrix (ECM) containing various proteins including elastin and fibrillar collagen types (I, III, V), non- fibrillar collagen (IV and VI), other glucose conjugates like sulfated proteoglycans and structural glycoproteins and glycosaminoglycans such as hyaluronate. The initial distribution matrix of these deposits occurs in the subendothelial Disse space area which is almost a basement membrane that creates an additional barrier in the spread between hepatocytes and sinusoids. Increased deposits of matrix then produce occlusion and disappearance of endothelial cell fenestrae. This phenomenon has become known as capillarization of sinusoids (7, 8, 13). The process of structural change in the matrix, deposits, and eventual formation of large fibrous septa, is an active process that takes a long time. It is greatly influenced by the activity of HSC, bile periphery, periportal fibroblasts, multiple growth factors and angiogenics. Certainly the type and persistence of the lesion, and its ability to degrade the matrix also exert influences as discussed below (4, 18, 20, 21).

Contractility

This feature of HSC is a very important factor in increases in portal resistance in early and late stages of liver fibrosis. It is presumably still reversible by thickening of the septa (22, 23). In early stages of fibrosis activated HSCs quickly show a phenotype similar to smooth muscle cells. This phenotype is characterized by increased contractile filaments including smooth muscle actin and myosin which generate forces which can be either independent of calcium or calcium-dependent (8,13). The acquisition of the contractile phenotype of HSC is mediated in part by receptors that interact with the extracellular matrix and which conduct calcium signals. Endothelin 1 is the main agonist which controls contractility in HSC, although there is a long list of other mediators including angiotensin II, vasopressin, eicosanoids, thrombin, and alpha adrenergic agonists (21). On the other hand, administration of endothelin 1 receptor antagonists and other agents including nitric oxide, carbon monoxide and prostaglandins, has induced reduction of portal pressure in portal hypertensive rats (21).

HSCs have been recognized as liver specific pericytes that contribute to the development and regeneration of the liver and its response to injuries. After a partial hepatectomy HSCs and endothelial cells migrate to vascular connections to establish new branches of sinusoids together with hepatocytes (22).

In advanced fibrosis the bands typical of end-stage fibrosis contain a large number of activated HSCs. They gradually restrict portal blood flow by constricting individual sinusoids and by contracting the cirrhotic liver. At the same time the density of HSCs and coverage of the lumen increases (22). The progressive development of intrahepatic shunts also requires an angiogenic response conducted by HSC, as already mentioned.

Release of cytokines

As mentioned HSCs are central modulators of liver inflammation and immunity and are not just passive subjects of a vast array of inflammatory cytokines. They have a regulatory role in the inflammatory response to lesions and the subsequent development of fibrosis (See text for the role of different cell types in activation of HSC) (13, 24).

Degradation of the extracellular matrix MEC (8, 13, 25-28)

While fibrosis reflects the balance between production and degradation of the matrix, it also constitutes a key event in hepatic fibrosis. The disruption of MEC in early stages of liver disease and the replacement of MEC by scar matrix may be referred to as pathological as can disruption of the normal liver matrix by tumor invasion or dysplasia. However, excess matrix resorption in patients with chronic liver disease is now seen as an opportunity to reverse liver dysfunction and portal hypertension. This is the result of the knowledge of matrix remodeling gained in recent years (25).

A major element in the matrix remodeling is a family of metalloproteinases known as matrixins. These are calcium dependent enzymes that degrade collagen and noncollagenous substrates. As a general rule metalloproteinases are classified into five categories according to substrate specificity.

1. Interstitial collagenases: matrix metalloproteinase (MMP) -1, -8, -13
2. Gelatinase: MMP -2, -9, fibroblast activating protein.
3. Stromelysin: MMP-3, -7, -10, -11.
4. Membrane Type: MMP-14, -15, -16, -17, -24, -25.
5. Metallo-elastases: MMP-12. (13, 26, 27)

HSCs are the main source of MMP-2, which increases in cirrhotic patients, and also MMP-9 and -13 and stromelysin. The main determinant of the progression of fibrosis is failure of degradation of the excess ECM production excess or of the matrix scar. HSCs produce small amounts of enzymes of the MMP-1family which can degrade Type I collagen, the main constituent of fibrotic liver. Regulation of the activity of matrix metalloproteinases may occur at various levels, but its inactivation occurs through binding to tissue inhibitors of metalloproteinase (TIMPs). HSCs produce TIMPs -1 and -2. Sustained production during hepatic injury may lead to matrix accumulation by inhibiting the activity of interstitial collagenase (13, 26-28).

RESOLUTION

The attention of researchers has been directed towards the issue of resolution because of its therapeutic potential. Research has established two routes by which HSC activation can reduce or reverse hepatic fibrosis:

1. Reversion to quiescent phenotypes
2. Clearance through apoptosis of activated HSC.

The first mechanism has not been validated in vivo, but while it is being investigated, research is accumulating a growing body of evidence supporting apoptosis as an important mechanism in fibrosis regression. HSCs present CD95.l mediated apoptosis and apoptosis induced by expression of the TNF-related apoptosis-inducing ligand (TRAIL). Natural killer cells (NK cells) induce the same mechanism as TRAIL does (29).

The antifibrotic role of NK cells is consistent with clinical findings showing that immunosuppression, as occurs in the use of cyclosporine and corticosteroids, increases liver fibrosis. This also explains how in the aging population when NK diminishes, fibrosis accelerates (30).

DIAGNOSIS OF FIBROSIS

Given that the objective of this review is not to discuss the methods used for diagnosis and grading of fibrosis, we will only mention some of the general concepts.

Liver Biopsy

The lack of a precise but noninvasive diagnostic method to validate the progression or regression of liver fibrosis is a major constraint for evaluation of anti-fibrotic effects of various therapeutic interventions. To date liver biopsy remains the highest standard available for the diagnosis of liver fibrosis/cirrhosis, but it is far from being a perfect gold standard (31-33). Based on the findings of Bedossa and others, there are at least three validated systems for diagnosis: the Ishak score, Desmet/Scheuer and the METAVIR system (in which F0 = no fibrosis while F4= cirrhosis) (33).

Among the main disadvantages of liver biopsy is the fact that it is an invasive method, not without risk, with a mortality rate of 1 in 10,000 (34). The interpretation of biopsy findings have significant variability in 20% of cases. Since the biopsy sample represents only a small part of the liver (1:50,000), a biopsy has the possibility of sampling error which can vary between 33% and 50% in both lobes according to a study of biopsy by laparoscopy in which samples taken from both lobes were found in one third of patients to have differences of at least one state between the two lobes (29, 30, 33, 35). Liver biopsy also increases the economic costs given that patients should be monitored for the risk of bleeding (34). For all the reasons mentioned earlier, people are daily trying to replace the liver biopsies with noninvasive methods such as serum biomarkers and elastography.

Biomarkers

These are intended to assess the state of fibrosis through measurement of proteins such as a2 macroglobulin, apo-A1, haptoglobin, hyaluronic acid and other liver enzymes. Batteries of tests available from laboratories include Fibrotest/Fibromax, Fibrosure, FibroSpect, Hepascore, and Fibrometer Fib 4 (36).

Problems associated with biomarker testing include high costs, availability, test contents, and heterogeneity. It is difficult to find diagnostic standards of accuracy in controlled and random clinical trials for validating the biostatistical techniques used in measuring biomarkers (36, 37). The Area Under the Receiver Operating Characteristic Curve test (AUROC) used to assess the performance of any alternative test is a binary hypothesis test which has significant data loss and depends on a certain state of fibrosis (28, 29). In addition, fibrosis is a category rather than a continuous variable (there are ranks in the accuracy of the biopsy and ranks in the prevalence of fibrosis. There is no linearity between the extent of fibrosis and histological stage. Thus the probability that the substitute (biomarker) accurately and correctly predicts the fibrotic condition of a patient is problematic (31, 32). Some meta-analyses (33, 35) show that there are alternative solutions with excellent values for identification of cirrhosis, but with less accuracy in the early and intermediate stages of fibrosis.

Finally, these elements need to be transferred to clinical practice given the remarkable enthusiasm that has been awakened for clinical trials of methods that might replace invasive tests. Flowcharts are now being tried that allow performance of liver biopsies in those clinical scenarios where non-invasive tests are not sufficiently powerful and stages F2 or F3 (the so-called gray areas) are suspected. Although these indications have not yet been precisely established, the possibility now exists that we will be able to determine the degree of fibrosis in a patient without doing a biopsy (31, 32, 38, 39, 40).

Elastography

Elastography is a noninvasive method for assessment of fibrosis that evaluates physical characteristics of the liver such as elasticity and stiffness (34). A thorough review of this method is outside the scope of this review, but we will mention its general characteristics.

This test uses ultrasound equipment (Fibroscan) that generates low frequency, low amplitude waves. By directing the sound waves through an intercostal space their velocity can be measured as they pass through the liver tissue. The results are reported in kilopascals (41). Technically, the elasticity of the liver is a measure of the force with which the tissue resists dimensional changes. It is quantified by the following formula which assumes that the liver is non-viscous, elastic and isotropic:

$$E = x\ V\ 3p^{2}$$

Where p is the mass density and v is the wave velocity (42).

Generally, ten measurements must be obtained for their average to be considered representative of hepatic elasticity. Obesity, ascites or a narrow intercostal space can result in an incorrect measurement. The test can be performed by nurses and paramedics after two weeks of training with 25 to 50 tests. Elasticity is measured at a depth of 25mm to 65 mm below the skin surface. The area measured is shaped like a cylinder with a diameter of 1 cm and a length of 4 cm. Intraobserver and interobserver agreement has been high with an intraobserver correlation coefficient of 0.96 to 0.98 and an interobserver correlation coefficient of 0.89 to 0.98.

A published meta-analysis suggests that this is an easy, useful, noninvasive and reproducible test. AUROC has high values for diagnosis of cirrhosis, with a 0.95 CI of 0.87 to 0.99 (38). However, even though it can handle a wide spectrum of elasticity values in advanced fibrosis states, it is less adequate for evaluating the differences between one grade and a higher grade of fibrosis (39, 42).

TREATMENT OF FIBROSIS

In recent years there have been an increasing number of enthusiastic reports about the reversibility of fibrosis. These are post-treatment studies in which the underlying disease is removed or eliminated. They include cases of eradication or inhibition of hepatitis B and hepatitis C viruses (1, 43-47). They also included cases of patients with autoimmune hepatitis who have responded to medical treatment with prednisone or an equivalent. Other patients who have shown this response are those with hemochromatosis whose fibrosis was reversed after iron depletion through phlebotomies. Among the potential therapeutic strategies for treating fibrosis are removal or treatment of underlying disease processes, treatment of inflammation that could lead to fibrosis, and treatment of HSCs by inhibiting activation, proliferation, contractility or fibrogenic response or by promoting apoptosis (1, 48). It is also possible to promote degradation of extracellular matrix.

The following are among the different drugs or strategies used to treat human patients.

Colchicine

Colchicine is an alkaloid derived from a plant. It inhibits microtubule polymerization which is a process required for the secretion of collagen. Apparently in experimental animal models it also inhibits synthesis, secretion and deposition of collagen. Taking into account its different actions and favorable safety profile, colchicine has been tested in several clinical circumstances with CBP on alcoholic cirrhosis. However, a recent review by Cochrane concluded that there is no evidence that it is superior to placebos in liver-related mortality, or in biochemical or histological improvement. Instead it was associated with increased side effects (RR 8.38, 95% CI: 1.08-65.2). The authors concluded that colchicine should not be used to treat fibrosis or alcoholic, viral or cryptogenic cirrhosis outside of clinical trials (45).

Pirfenidone

Pirfenidone is a small molecule which can be administered orally. It is an effective anti-fibrotic and anti-inflammatory that inhibits collagen synthesis by inhibiting tissue growth factors (41). In a small study of patients with hepatitis C and liver fibrosis that were treated with 1200 mg/day for twelve months, fibrosis was reduced by 30%. An assessment was by liver biopsy. Inflammatory indices improved 53% (46).

Peroxisome proliferator-activated receptors (PPARs)

These drugs which are used as oral agents to improve insulin resistance belong to the thiazolidinedione group. Particularly their second generation has been used to treat non-alcoholic steatosis-hepatitis (47). Their effects on liver histology particularly target steatosis and fibrosis. The PPAR group of drugs acts on the nuclear hormone receptors, with three groups known as alpha, beta and gamma or delta (45, 46). The PPAR gamma is expressed strongly in adipose tissue and HSCs (18, 48). In adipose tissue it regulates lipid metabolism and differentiation of adipocytes. In HSCs it causes decreases in transcriptional activity with activated phenotypes reverting to their quiescent states (18). A small pilot study of rosiglitazone found improvement in hepatocellular ballooning and perisinusoidal fibrosis in zone 3. 30 subjects who had histological evidence of NASH were studied for 48 weeks (49). However, another study with pioglitazone showed that post-treatment improvement was not maintained as measured in histology and/

or liver function tests (50). These small studies suggest the possibility of achieving histological improvement in these patients, but further studies with larger numbers of patients to validate these findings are needed (51).

Angiotensin II Receptor Antagonists

The renin-angiotensin system is a key mediator in regulating blood pressure and body fluid homeostasis. It also regulates the local hemodynamics of various organs (52). This system is particularly active in the liver in patients with liver cirrhosis who have increased levels of angiotensin II (ATII) and greater activation of HSCs and fibrogenesis (7, 8, 48). ATII is a vasoactive cytokine that induces portal hypertension, activation, contraction and proliferation of HSCs (7, 8, 48, 53). Its biological effects are the result of activation of different receptors, especially type 1 (AT1-R), generally with activities opposed to type 2 receptors (AT2-R) (52, 53). Its signals are then mediated intracellularly by NADPH oxidase which produces reactive oxygen species (52, 53). Inflammatory events are triggered inducing conformational changes in the flavocytochrome b which supplement electronic transfer from flavin adenine dinucleotide (FAD) to nicotinamide adenine dinucleotide NAD+ and superoxide anion. These are directly involved in the production and over-regulation of collagen alpha I gene expression (53). ATII also increases expression of TGF Beta and synthesis of extracellular matrix proteins (52, 53). Based on this knowledge agents which convert enzyme inhibitors or angiotensin receptor blockers have been studied experimentally as agents to counter fibrotic portal hypertension (53). Some clinical evidence suggests that the use of AT1 receptor antagonists significantly improves fibrosis in patients with chronic hepatitis C and NASH (54). A comparative study of 30 patients with hepatitis who received either losartan and ursodeoxycholic acid or ursodeoxycholic acid alone (55) found a slight decrease of ALT levels in the losartan group with no change in other liver function tests or levels of RNA-HVC. The levels of collagen type IV and FCGT B1 in the losartan group were significantly lower than those of the control group ($p < 0.05$). There were no differences in the METAVIR scores (55). In another study (56), 14 patients with chronic hepatitis C were selected to receive either 50 mg of losartan daily and then compared to an untreated group of 9 patients for a 6 month period. Changes in the state of fibrosis were significantly different for the losartan group (decrease of 0.5 + _1.3) and the control group (increase of 0.89 + / - 1.27) $p < 0.03$. In the treated group the fibrosis of 7 out of 14 patients diminished, while it decreased in only 1 out of 9 patients in the control group ($p<0,04$). Assessment by digital imaging also found less subendothelial fibrosis in the losartan group after treatment. Reduction in systolic blood pressure was observed, but there were no affects on mean pressure or renal function (56). These experiences suggest that blocking angiotensin II receptor may have anti-fibrotic effects although further studies are needed with larger numbers of patients.

Interferon

The interferon family consists of three major isoforms: alpha, beta and gamma (A, B, G). A and B isoforms share the same receptor and may leave more potent antiviral effects than the G isoform. Nevertheless, the interferon G has shown specific inhibition of ECM synthesis in fibroblasts, and in preclinical studies it has been shown to have multiple effects on HSC activation (57). The largest study so far (51) was a double-blind multicenter placebo-controlled study which used Interferon Gamma 1 B in 488 patients with Ishak fibrosis. Scores of 4-6 were assigned to each of 3 treatment groups: 100mg 1b range INF (Group 1 n = 169), 1b range INF 200mg (group 2 n = 157) and placebo (group 3 n = 162). There was no improvement of fibrosis in any of the three groups (57). In contrast, another study of 99 hepatitis B patients found that fibrosis scores improved by 63% in the group treated with INF-gamma, compared to 24% in the placebo group (58). These contradictory results merit further studies to determine the potential effectiveness of this medicine.

Herbal medicines

There is no evidence regarding the effects of these substances on human liver fibrogenesis. Among the substances used, especially in mainland China, are those that contain salvia and salvianolic acid B. This is a water soluble phenolic acid with effects on HSCs (48). Some other substances proposed as antifibrotics are cucurmin, glycerine, celasterol, tetrandrine, berberine and oxymatrine (59). However, because of their significant toxicity, including in the liver, clinical use requires extreme caution (48, 59).

Other strategies

New approaches and strategies currently address several areas and systems. These approaches are based on the understanding of the different fibrogenic pathways involved. The most important one involves FTC beta and attempts to inhibit this cytokine to interrupt the fibrotic process.

It is seen as a promising antagonist to cannabinoid receptors CB1 and CB2, presumably working through inhibition of FTC beta 1 expression and inhibition of hepatic myofibroblast growth (60).

Inhibition of angiogenesis mechanisms, interruption of intracellular signals and interruption of nuclear transcription have been found to be attractive antifibrotic mechanisms (1, 48). The use of KB kinase inhibitors with sulfasalazine as well as interference with small RNA signals (micro RNA signals) that play important role in fibrogenesis are of particularly great interest as potential activators of apoptosis of HSCs (60-62).

Conflicts of interest

None.

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