Insulin-Like Growth Factor-1 Deficiency and Cirrhosis Establishment

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Abstract

Cirrhosis represents the final stage of chronic liver damage, which can be due to different factors such as alcohol, metabolic syndrome with liver steatosis, autoimmune diseases, drugs, toxins, and viral infection, among others. Nowadays, cirrhosis is an important health problem and it is an increasing cause of morbidity and mortality, being the 14th most common cause of death worldwide. The physiopathological pathways that lead to fibrosis and finally cirrhosis partly depend on the etiology. Nevertheless, some common features are shared in this complex mechanism. Recently, it has been demonstrated that cirrhosis is a dynamic process that can be altered in order to delay or revert fibrosis. In addition, when cirrhosis has been established, insulin-like growth factor-1 (IGF-1) deficiency or reduced availability is a common condition, independently of the etiology of chronic liver damage that leads to cirrhosis. IGF-1 deprivation seriously contributes to the progressive malnutrition of cirrhotic patient, increasing the vulnerability of the liver to establish an inflammatory and oxidative microenvironment with mitochondrial dysfunction. In this context, IGF-1 deficiency in cirrhotic patients can justify some of the common characteristics of these individuals. Several studies in animals and humans have been done in order to test the replacement of IGF-1 as a possible therapeutic option, with promising results.

Keywords: IGF-1; Steatosis; Non-alcoholic fatty liver disease; Acute liver damage; GH/IGF-1 axis; Fibrogenesis; Oxidative damage; Mitochondrial protection

Introduction

Cirrhosis is the result of chronic liver disease. It represents the final stage of a wide number of chronic liver conditions, whose common effect is necroinflammation, fibrosis and regeneration nodules that modify the normal liver structure reducing its functional mass and altering the vascular liver architecture [1-3]. Fibrosis progresses at variable rates depending on the cause of liver disease, environmental and host factors [4-6]. However, all those structural liver changes lead to impaired hepatocyte function (hepatocellular insufficiency) and an increased intrahepatic pressure (portal hypertension) leading to all the clinical manifestations in cirrhosis [7].

The transition from chronic liver disease to cirrhosis involves inflammation, and activation of hepatic stellate cells (HSCs) leading to fibrogenesis - angiogenesis and parenchymal lesions - partly due to vascular occlusion (Fig. 1) [8]. These changes produce hepatic microvascular rearrangement, such as sinusoidal remodeling, formation of intrahepatic shunts, and hepatic endothelial dysfunction [9]. The endothelial dysfunction alters the normal release of vasodilators, most importantly nitric oxide. In addition, there is an increased production of vasoconstrictors [10]. All these changes, in combination with structural disturbances generate increased hepatic resistance to portal blood flow, leading to an elevated portal pressure, with its clinical consequences.

Furthermore, recently our group has described that the mere insulin-like growth factor-1 (IGF-1) partial deficiency, in an animal model with such deficiency, is associated with relevant alterations of hepatic architecture and expression of genes involved in cytoskeleton, hepatocyte polarity, cell junction and extracellular matrix (ECM) proteins [11]. These results suggest a novel approach to overcome the physiopathology in the onset of liver damage, IGF-1 availability, IGF-1 receptor expression after injury and cirrhosis development, claiming further investigation. To date, such results have not been studied in humans.

Cirrhosis is a dynamic process that has to be monitored frequently, in order to avoid progression and/or reverse fibrosis [12, 13]. It can remain in a compensated state for several years, but when progression persues to advanced stages, complications may appear leading to a poor quality of life with higher morbidity and mortality. In advanced stages, the most common complications include coagulopathy and jaundice, gastrointestinal bleeding from esophageal varices, ascites, hepatorenal syndrome, spontaneous bacterial peritonitis, encephalopathy, hipogonadism, and malnutrition [14, 15].

Nowadays, when treating cirrhotic patients, the aim is to avoid or delay progression to a “decompensated” stage, where
mortality rises up to 85% over 5 years, and to avoid liver transplantation [7]. Nevertheless, drug therapies can partially prevent or control some complications, but none of them can significantly increase survival nor modify the natural clinical course of the disease, except for some etiologic specific therapies.

For this reason, new therapies have been tested in order to modify the natural history of cirrhosis, improving hepatocellular function and reducing portal pressure. In this context, it seems necessary to improve our knowledge in the early onset of liver disease as well as its transition to cirrhosis, in order to find different therapeutic alternatives. This approach will be reviewed in the following lines.

Epidemiology

Cirrhosis is an increasing cause of morbidity and mortality, being the 14th most common cause of death worldwide [7]. Its prevalence is difficult to assess because the initial stages of the disease are asymptomatic, thus remaining undiagnosed, and is probably higher than reported.

In a recent epidemiologic study of cirrhosis, global liver cirrhosis deaths represented more than one million in 2010, or 1.95% of the global total deaths. On average, there were twice as much male deaths compared to women [16].

Furthermore, most Western European countries have improved its cirrhosis mortality, with the exception of UK, Ireland and Finland, where cirrhosis mortality rates have been increasing since 1980 [16]. In Latin America, mortality rates vary among different countries. Mexico has remained the country with the highest cirrhosis mortality rate in the region (Latin America), at 38.3 (30.7 - 47.5) per 100,000, and in 2010, it was the fourth leading cause of death, accounting for 18% of deaths in males aged 40 - 49 years [16]. Concerning Central Asia, the mortality rates have increased since 1990s until 2000, but in the last decade, the mortality rate has decreased or stabilized in these countries. Finally, in sub-Saharan Africa, cirrhosis deaths have been doubled between 1980 and 2010 [16].

Alcoholic liver disease and hepatitis C are the main causes in most developed countries, while hepatitis B is the most common cause in most parts of Asia and sub-Saharan Africa (Table 1) [3, 16].

Moreover, it is well known that metabolic syndrome (MetS) is increasing worldwide [17], in part related to the occidentalization of lifestyle habits [18, 19]. Non-alcoholic liver disease is also increasing in developed countries [20, 21]. It is important to take this into account, as MetS represents a major cause of non-alcoholic steatohepatitis (NASH) and non-alcoholic fatty liver disease (NAFLD). Of interest, accumulated evidence relates IGF-1 deficiency with MetS establishment and steatohepatitis, since the mere IGF-1 deficiency alters hepatic expression of gene involved in glucose and lipid metabolism [22].
Growth Hormone (GH)-IGF-1 Axis, IGF-1 Deficiency, Altered Lipid Metabolism and Oxidative Damage

IGF-1 is a 70-aminoacidic anabolic hormone with several endocrine, paracrine and autocrine effects [23]. It is well known that IGF-1 is mainly produced by the liver (accounting for 75% of circulating IGF-1), but almost every tissue is able to secrete IGF-1 for autocrine/paracrine purposes [24-27]. Pituitary GH and liver derived IGF-1 establish a negative feedback mechanism (Fig. 2) [28-30].

Circulating IGF-1 can be found in its free form or mainly bound to carrier proteins (IGF binding proteins (IGFBPs)). Because IGF-1 has a wide range of physiological roles, it must be strictly controlled, where IGFBPs play an important role. Until now, there have been identified at least six high affinity IGFBPs. IGFBP-3, which binds 90% of circulating IGF-1, forms a ternary complex consisting of one molecule of IGF-1, one molecule of IGFBP3 and one molecule of the so-called acid labile subunit [31]. GH mainly regulates the IGFBP-3 [32], while IGFBP-1 is mostly regulated by insulin and IGF-1 [33].

In summary, the common functions of IGFBPs are: 1) acting as carrier proteins for circulating IGF-1 and controller of its flow from the vascular space to tissues; 2) increasing IGF-1 half-life and regulating its metabolic clearance [34]; 3) modulating the interaction between IGF-1 and its receptor, and thus indirectly controlling IGF-1 biological activity [29]; 4) modulating IGF-1 in target tissues, inhibiting or activating its specific actions: cell proliferation, differentiation, survival and migration [28, 35-37]; and 5) providing a specific localization pool of IGF-1, because IGFBPs can associate with cell membranes or ECM [38]. Additionally, some IGFBPs may have some biological effects outside IGF-1 signaling pathways, such as apoptosis induction, and proliferation/inhibition in some tumors [37].

Additionally, other nine binding proteins arose as IGFBP-related proteins (IGFBP-rPs), with structural and functional similarities to the IGFBPs but with lesser affinity [34]. Nonetheless, the physiological role of these proteins in the IGF system is not completely defined, but their structural relationship with IGFBPs allows some of these proteins to bind IGF-1, controlling its activity [39, 40].

IGF-1 actions are mediated by its binding to its putative receptor, IGF-1R, a tyrosine kinase. Nonetheless, IGF-1 can also bind to the insulin receptor (with lower affinity), regulating some metabolic functions. Type 1 IGF receptor is a heterotetramer composed of two extracellular α subunits and two transmembrane β subunits. The extracellular α subunits are cysteine-rich regions that confer specificity to ligands, while β subunits have an intracellular part that contains a tyrosine kinase domain, which constitutes the signal transduction mech-

Table 1. Population Fractions for Liver Cirrhosis Risk Factors by Region in 2010

| Region name            | Alcohol | Hepatitis B | Hepatitis C | Other*  |
|------------------------|---------|-------------|-------------|---------|
| Asia Pacific, high income | 0.24    | 0.31        | 0.25        | 0.20    |
| Asia, Central          | 0.16    | 0.36        | 0.18        | 0.29    |
| Asia, East             | 0.18    | 0.39        | 0.18        | 0.26    |
| Asia, South and Southeast | 0.40    | 0.58        | 0.44        | 0.59    |
| Australia              | 0.31    | 0.30        | 0.18        | 0.21    |
| Caribbean              | 0.25    | 0.14        | 0.25        | 0.36    |
| Europe, Central        | 0.27    | 0.15        | 0.22        | 0.36    |
| Europe, Eastern        | 0.30    | 0.13        | 0.23        | 0.34    |
| Europe, Western        | 0.33    | 0.11        | 0.30        | 0.27    |
| Latin America, Andean  | 0.23    | 0.21        | 0.21        | 0.36    |
| Latin America, Central | 0.29    | 0.08        | 0.26        | 0.37    |
| Latin America, Southern | 0.31   | 0.12        | 0.28        | 0.29    |
| Latin America, Tropical | 0.31  | 0.06        | 0.27        | 0.37    |
| North America, high income | 0.33  | 0.06        | 0.29        | 0.32    |
| North Africa, Middle East | 0.14  | 0.27        | 0.24        | 0.36    |
| Sub-Saharan Africa, Central | 0.15 | 0.37        | 0.20        | 0.27    |
| Sub-Saharan Africa, East | 0.16  | 0.34        | 0.20        | 0.30    |
| Sub-Saharan Africa, Southern | 0.19 | 0.37        | 0.18        | 0.27    |
| Sub-Saharan Africa, West | 0.15  | 0.38        | 0.18        | 0.28    |
| Oceania                | 0.13    | 0.44        | 0.17        | 0.26    |

*Not attributable to chronic alcohol intake, and tested negative to anti-VHC antibodies and HbsAg. Adapted from Mokdad et al, BMC Medicine 2014;12:145.
IGF-1 Deficiency and Liver Disease

Tyrosine phosphorylation activates a signaling cascade [41]. IGF-1 has paracrine, endocrine, and autocrine effects on almost every organ, owing this fact to the ubiquitous IGF-1R expression in the organism [30].

The relevance of IGF-1, both in embryological and postnatal states, has been known for years, and its important role in multiple organs has gained recognition more recently. Its wide activities are partly summarized in Figure 3 [22, 43-68].

Recent data also support that IGF-1 deficiency is related to insulin resistance, impaired lipid metabolism, oxidative damage and neuro-hormonal axis deregulation [69-71]. Likewise, some studies have also suggested an inverse relationship between IGF-1 circulating levels and the incidence of MetS, with liver steatosis, insulin resistance, hyperlipidemia and abdominal visceral obesity [61, 72-75]. All these results suggest a possible major role of IGF-1 in the development of MetS as well as NASH and NAFLD, which constitute, in many cases, the first stage of metabolic liver damage.

Physiopathological Pathways Since Early Liver Damage to Decompensated Cirrhosis: Relationship With IGF-1 Deficiency

The beginning of fibrosis is usually insidious and the progression to cirrhosis can occur in an interval of 15 - 20 years, depending upon different factors - etiology, genetic and environmental aspects [76]. Even though each etiology has its specific pathological feature, a general common pathway can be described from early liver damage all the way to cirrhosis. Hepatic fibrosis is the result of the liver’s response to a repeated injury that can be due to viral infection, MetS with insulin resistance, autoimmune disease, toxins, or alcohol [77]. After an acute injury, the hepatocytes become damaged and an inflammatory response is triggered following HSCs activation, leading to a controlled and coordinated deposition of ECM with parenchymal cells regeneration and replacement of necrotic cells. If the injury persists, this regeneration process fails, and ECM replaces the normal liver parenchyma, through the proliferation and differentiation of HSC to myofibroblasts. Those myofibroblasts secrete different profibrogenic cytokines that finally lead to the synthesis and deposition of fibrillar collagen that forms the ECM. Sinusoidal endothelial cells loose their fenestrations and deposition of ECM increases the resistance to hepatic blood flow. A positive feedback is then established, in which inflammatory and profibrogenic cells stimulate each other, leading to fibrosis, with accumulation of ECM because of the increased synthesis and decreased degradation, as well as the increasing resistance to hepatic blood flow [76].

A common feature that has been described occurring from the early stages of liver damage, central for NAFLD and
NASH, is IGF-1 deficiency [78-80]. There are two important aspects of this issue. 1) In both cases (NAFLD and NASH), the insulin resistance plays a major role in the development of liver damage, even though different factors are also implicated (obesity, type 2 diabetes, MetS, and hyperlipidemia, among others) [78]. It is well known that IGF-1 improves insulin sensitivity in vivo, and also that the specific deletion of hepatic IGF-1 results in insulin resistance [81], showing that hepatic IGF-1 regulates systemic insulin sensitivity. 2) As previously mentioned, some recent studies suggest a relationship between IGF-1 deficiency and the risk of developing MetS [61, 82-84], which further would contribute to the appearance of NAFLD and/or NASH.

In conclusion, NAFLD and NASH could be a later manifestation of an IGF-1 deficiency condition, as this deficiency contributes to the presence of different risk factors that lead to the aforementioned diseases. Moreover, IGF-1 deficiency has been described in both entities, so it could represent a common pathway between MetS and hepatic steatosis [78] (Fig. 4).

Numerous different factors take place in this complicated mechanism that can either enhance or ameliorate the activated fibrogenic cascade. A complex interplay between different hepatic cells takes place, leading to the release of reactive oxygen species (ROS) together with fibrogenic and inflammatory mediators, among others. There is growing evidence for the contribution of different immune interactions, chemokines, adipokines, oxidative stress and neuroendocrine factors [76, 77]. Table 2 resumes the inflammatory mediators that are involved in the regulation of fibrogenesis in the liver [85]. These mediators function independently of IGF-1 levels.

The oxidative stress, mitochondrial dysfunction, and inflammatory cascade, also play an important role in the development and persistence of liver damage of any etiology, leading to fibrosis [76, 77]. Nowadays, it is better understood that all these mechanisms interact with each other promoting fibrosis, and, should they persist activated, the damage will ensue, contributing to cirrhosis establishment and progression. Nevertheless, the cessation of liver injury has been confirmed to delay and even revert fibrosis at some degree, although this may take several years [77]. In this sense, recent studies focus on the investigation of possible therapeutic targets that could act directly in each profibrogenic pathway, in order to stop this negative stimuli and hence delay or revert fibrosis. However, poor results have been obtained so far.

Despite this, some studies have shown that IGF-1 reduces oxidative stress in the liver, and improves mitochondrial function [86, 87]. Also, it has been described that GH-deficient rats present impaired mitochondrial morphology of the hepatocytes, which improves with IGF-1 administration [88]. As these factors are strongly implicated in the progression of liver damage, it can be a useful target for new therapeutic approaches. In our experience, mitochondria is one of the main intracellular targets of IGF-1, proved in several conditions of IGF-1 deficiency and restored by low doses of this hormone [87].

Moreover, as previously mentioned, it has been recently described that the mere IGF-1 partial deficiency in animals is associated with relevant alterations of the hepatic architecture, as well as an altered expression pattern of genes encoding cytoskeleton proteins, genes related to hepatocyte polarity, cell
junctons and ECM proteins, suggesting that IGF-1 deficiency can be strongly implicated since the early stages of liver damage. Additionally, this partial deficiency induced an altered liver expression of genes encoding IGF-1R and proteins involved in acute-phase and inflammation, resulting in hepatic oxidative damage. Also, our group described that cirrhotic animals showed a significant reduction in IGF-1 circulating levels, that IGF-1 treatment restored to normal [89].

Considering all these data, it can be suggested that early liver damage and cirrhosis are IGF-1 deficiency conditions that can be improved with IGF-1 treatment, as the liver expresses IGF-1R under both conditions [11].

**IGF-1 and GH in Cirrhosis**

Liver cirrhosis association with IGF-1 was first described in the late 80s, when it was proposed as a good marker of hepatocellular function. Since then, the idea of liver cirrhosis as a condition of IGF-1 deficiency has been consolidated over the last years.

As previously mentioned, decreased levels of free IGF-1 are observed in patients with chronic liver disease (becoming more severe as the disease progresses) [90-94], despite the normal or elevated GH secretion [91, 95]. This may be due to a decrease in GH receptors on the liver of these patients [96-98], and a progressive reduction of liver synthesis capability. Likewise, IGFBPs production is also modified in cirrhosis, with an increased level of IGFBP-1 and a decreased of IGFBP-3. These changes may play a special role in the bioavailability of IGF-1 in tissues [94, 99, 100], since IGFBP-3 carries up to 80-90% of circulating IGF-1 and maintains plasma concentrations, meanwhile IGFBP-1 sequesters IGF-1, impeding its usage (Fig. 5).
| Tipo                     | Mediator | Target cells and mechanisms of action                                                                                                                                                                                                 | Liver disease/model                                                                 |
|-------------------------|----------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------|
| Inflammatory cytokines  | IL-1     | Up-regulates TIMP-1 and down-regulates BAMBI in HSCs. Promotes HSC survival. Promotes lipid accumulation and cell death in hepatocytes during NASH and ALD.                                                                                     | Experimental fibrosis induced by BDL or TAA; experimental NASH by CDA diet; experimental ALD model induced by Lieber-DeCarli and ethanol binge injection. |
|                         | IL-33    | Secreted from damaged hepatocytes, stimulating ILC2 to produce IL-13 that in turn activates HSC.                                                                                                                                                                                                   | Human liver cirrhosis; experimental fibrosis induced by CCl₄, TAA or Schistosoma mansoni infection. |
|                         | TNF-α    | Induces apoptosis of the hepatocytes. Up-regulates TIMP-1 and down-regulates BAMBI in HSCs. Promotes HSC survival and proliferation. Activates liver macrophages.                                                                                                                                     | Experimental fibrosis induced by BDL; experimental NASH model induced by MCD diet.       |
|                         | IL-17    | Stimulates KCs and HSC to produce IL-6, TNF-α, and TGF-β. Activates NF-kB and STAT3 in KCs and HSCs. HSCs activation through STAT3.                                                                                                              | Hepatitis B, experimental fibrosis induced by CCl₄ or BDL.                              |
|                         | IL-20    | Promotes activation, proliferation, and migration of HSCs. Prevents hepatocyte injury.                                                                                                                                                                                                               | HBV- and HCV-induced liver cirrhosis; experimental fibrosis induced by CCl₄.            |
|                         | IL-22    | Induces HSC senescence through STAT3-p53. HSC senescence inhibits liver fibrosis.                                                                                                                                                                                                                    | HBV-, HCV- and alcohol-induced liver cirrhosis; experimental fibrosis induced by CCl₄. |
|                         | IFN-γ    | Suppresses HSC proliferation and activation. Activates NK cells to promote HSC killing.                                                                                                                                                                                                               | Experimental fibrosis induced CCl₄.                                                   |
| Chemokines              | CCl₂ (MCP-1) | Macrophage and HSC recruitment; HSC activation.                                                                                                                                                                                                                                                   | Experimental fibrosis induced by CCl₄ or BDL; experimental NASH model induced by MCD or CDA diet. |
|                         | CCL5     | Macrophage and HSC recruitment; HSC activation.                                                                                                                                                                                                                                                     | Experimental fibrosis induced by CCl₄.                                                 |
|                         | CXCL9    | Suppresses HSC activation. Inhibits angiogenesis that inhibits liver fibrosis.                                                                                                                                                                                                                       | Experimental fibrosis induced by CCl₄.                                                 |
|                         | CXCL10   | Promotes hepatocyte death and HSC activation. Inhibits NK cell-mediated HSC inactivation.                                                                                                                                                                                                            | Experimental fibrosis induced by CCl₄.                                                 |
|                         | CX3CL1   | Prolongs KC survival. Promotes anti-inflammatory property in KCs.                                                                                                                                                                                                                                   | Experimental fibrosis induced by CCl₄ or BDL.                                        |
| Gut microbiota axis/TLR pathway | TLR4       | Directly stimulates HSC to down-regulate BAMBI and produce chemokines in BDL and CCl₄-induced liver fibrosis. Stimulates KCs to produce proinflammatory and fibrogenic cytokines that activate HSCs in ALD and NASH. Stimulates LSECs to induce angiogenesis that promotes HSC activation and fibrosis. | Experimental fibrosis induced by CCl₄ or BDL; experimental NASH model induced by MCD or CDA diet; experimental ALD model induced by Lieber-DeCarli or Tsukamoto-French model. |
|                         | TLR2     | Stimulates KCs to produce cytokines that activate HSCs in NASH. Stimulates macrophages in intestine, which promote bacterial translocation.                                                                                                                                                      | Experimental fibrosis induced by CCl₄ or BDL; experimental NASH model induced by CDA diet. |
|                         | TLR9     | Stimulates KCs to produce cytokines that activate HSCs in NASH. Stimulates HSCs by host DNA released from apoptotic Hepatocytes.                                                                                                                                                                      | Experimental NASH model induced by CDA diet; experimental fibrosis induced by CCl₄ or BDL. |
|                         | TLR3     | Stimulates NK cells to produce IFN-c that induces antifibrotic effect by killing HSCs.                                                                                                                                                                                                                 | Experimental fibrosis induced by CCl₄ or Lieber-DeCarli plus CCl₄.                     |
|                         | TLR7     | Stimulates DCs to produce type I IFN that inhibits liver fibrosis.                                                                                                                                                                                                                                   | Experimental fibrosis induced by CCl₄ or BDL.                                        |

Adapted from Seki et al, Hepatology, 2015.
On the other hand, the hepatocytes express few IGF-1 receptors in healthy subjects, so it has been thought that IGF-1 may not affect hepatocyte function directly. However, IGF-1R overexpression in hepatocytes has been described in chronic hepatitis C, chronic hepatitis B, and liver cirrhosis [101-103], when compared with normal livers, suggesting the potential role of IGF-1 in the liver damage under these conditions [104]. Additionally, recent results of an experimental model of IGF-1 partial deficiency induced the expression of IGF-1R in the liver, even though no liver injury is present. So, IGF-1 seems to play an important role in liver homeostasis.

Some other experimental models have shown the physiological importance of GH signaling in the liver. Liver-specific deletion of GH receptor in mice (GHRLD) resulted in a reduction of >90% of serum IGF-1 levels, contributing to those previously discussed effects [105]. Furthermore, these mice also showed insulin resistance, glucose intolerance, increased fatty acids, decreased triglyceride efflux, severe steatosis, as well as impaired liver regeneration, which proposes that GH may regulate hepatocyte proliferation [65, 106, 107].

Under this scenario, several characteristics of cirrhotic patients can be partially justified by IGF-1 deficiency, such as malnutrition. Glucose production through liver gluconeogenesis is increased in these patients, as well as proteolysis in the muscle. Likewise, they exhibit an increase in insulin and glucose levels partly secondary to insulin resistance, but the exact mechanism is still not well understood. Several causes have been proposed so far, but none of them have been yet confirmed [29, 99, 108]. Additionally, low IGF-1 levels contribute to the loss of bone mass seen in cirrhotic patients [109].

Results of IGF-1 Therapy

Results of low doses of recombinant human IGF-1 (rhIGF-1) administration in experimental models of cirrhosis

Following the consolidation of cirrhosis as a condition of IGF-1 deficiency, the replacement therapy with this hormone has been approached in multiple studies in order to test it, given its possible role in the genesis of some cirrhosis complications. Some of these studies results are discussed below.

Experimental CCl4-induced cirrhosis animals have been treated with rhIGF-1 (20 μg/kg/day for 14 - 21 days) versus placebo, showing the following results: 1) Increased food ingestion, nitrogen balance and the uptake of dietary nitrogen by muscle, leading to increased muscle mass [110, 111]. 2) In vivo and in vitro studies showed recovery of cirrhosis-associated micro-villi atrophy [111-113], as well as carbohydrates and amino acids intestinal absorption, but no lipid absorption recovery [49, 70, 112, 114, 115]. The intestinal absorption improves as morphologic changes do [115]. 3) Increase of bone density and reduced bone resorption, improving osteopenia, both in compensated and ascitic cirrhosis [116]. 4) Reversion of testicular atrophy as well as histological alteration and improvement of hypofisisis-testicular axis. Hemato-testicular
Since the administration of rhIGF-1 is extremely expensive, it has been proposed that the use of viral vectors encoding IGF-1 can allow sustained expression of the transgene within the cirrhotic liver, helping in treating cirrhosis instead of rhIGF-1 administration [126, 127]. Two studies have been done so far to evaluate this possibility, and Table 3 summarizes the different findings [128]. In the first model, a recombinant simian virus 40 (rSV40) vector encoding for IGF-1 was used to evaluate if the sustained expression of IGF-1 in the liver can protect it against developing cirrhosis after a chronic exposure to CCl4. It was found that rSV40 encoding IGF-1 reduced liver fibrosis score) [69]. Furthermore, a decrease in collagen mRNA expression has been also described [50]. Additionally, an improvement in liver function, an increase in albumin and coagulation factor levels, and a reduction in bilirubin levels were found [69]. Some of these changes have been also demonstrated in cirrhosis induced by common bile duct ligation.

6) Regularization of mitochondrial function in the liver [69, 86, 87, 120] and ATPase function, as well as decrease in oxidative stress parameters and free radicals [69]. In particular, a normalization of mitochondrial membrane potential, an increase of ATP production, reducing the intramitochondrial free radical production, as well as a decrease caspase activation and apoptosis have been described [87].

In summary, IGF-1 replacement therapy shows several hepatoprotective, antifibrogenic, anti-inflammatory, and antioxidant effects.

Other changes show decrease in lipid peroxidation products and free radicals, decreasing the collagen gene expression in myofibroblasts [121] and prolyl-hydroxylase activity [122], as well as stellate cells activation [123]. Furthermore, the expression of several genes that were altered in CCl4-induced cirrhosis, was normalized after rhIGF-1 replacement therapy [124]. Additionally, the regenerating activity increases with the proliferation of cell nuclear antigen expression, the restoration of GH receptor gene expression, and the stimulation of hepatocyte growth factor production (a potent mitogen and liver protecting agent), as well as the down-regulation of transforming growth factor-β1 (TGF-β1) [125].

### Results of rhIGF-1 administration in cirrhotic patients

In human cirrhotic patients, one clinical trial has been conducted, being a pilot, double-blind, randomized, placebo-controlled study in order to evaluate the effects of rhIGF-1 administration in patients with primary biliary cirrhosis or alcohol-related cirrhosis [129]. During 4 months, patients received rhIGF-1 in the following manner: initially 20 μg/kg/day, increasing the dose each week to a maximum dose of 50 μg/kg/day or 100 μg/kg/day for 4 weeks. Even with the limitations of the study, there were three main findings in the patients receiving rhIGF-1: 1) an increase in serum albumin levels, which has never been achieved by any other treatment, 2) a trend towards increased resting energy expenditure and total IGF-1, which can be due to an increased amount of available ATP, because of an improvement of mitochondrial function [87], and 3) an augmented IGF-1/IGFBP3 ratio levels. In this study, IGF-1 was well tolerated and more effective in patients with less nutritional impairment, higher hormone bioavailability rates, and

### Table 3. Factors Up- and Down-Regulated After IGF-1 Gene Transfer in Cirrhotic Patients (Modified From Bonefeld and Moller, Liver Int. 2011)

| Up-regulated hepatoprotective factors | Down-regulated profibrogenic factors |
|--------------------------------------|--------------------------------------|
| HGF       | Activated HSC                      |
| MMPs      | αSMA                               |
| HNF4α     | TGF-β                               |
| STAT3a    | STAT3b                             |
| Egfr      | TIM1 and TIM2                      |
| Hnf6      | PDGF                               |
| Prlr      | CTGF                               |
| Lifr      | WT-1                               |

Same factors could be involved in the positive clinical outcome seen when supplementing with rhIGF-1.

### Results of IGF-1 gene transfer in experimental models

Since the administration of rhIGF-1 is extremely expensive, it
those with alcoholic cirrhosis.

**Other Strategies for Cirrhosis Treatment**

The progression in understanding the pathophysiological mechanisms of cirrhosis has generated new investigations about possible therapeutic drugs that can prevent, delay or reverse fibrosis. Nowadays, the approach to liver fibrosis can be divided into two steps: primary therapy, in order to prevent, delay or even revert fibrosis, comprising in treating the underlying cause (hepatitis B, C, autoimmune hepatitis, alcohol consumption, etc.) [130-134]; secondary therapy, in order to revert fibrosis developing intrinsic antifibrotic drugs that target the fibrogenesis mechanism. In this scenario, many drugs have been tested so far in experimental animals, but clinical tests of some of them are still pending [76, 135-137]. Moreover, some drugs have shown convincing antifibrotic activity on HSCs in vitro, as well as in animal models of liver fibrosis and even patients in vivo [76, 135-137]. Nevertheless, their long-term safety in cirrhotic patients has not been proven to date.

On the other hand, the reconstitution of functional parenchymal mass in conjunction with fibrosis treatment can lead to a better prognosis [138-140]. In this aspect, hepatocyte transplantation as well as infusion of hepatocyte growth factor, has shown to improve liver function [141, 142]. Moreover, the transplantation of hepatocyte stem cells or progenitor cells promises a better future in the treatment of cirrhotic patients. However, until now, the efficiency of these approaches is still very low, needing further investigations in order to improve the techniques, so they can be applied to patients.

**Conclusions and Future Perspective**

Recently, our knowledge about cirrhosis development and evolution has increased. IGF-1 seems to play an important role in the development and progression of this condition, being a possible marker of the functional reserve of hepatocellular functional capacity [90, 143]. Furthermore, some studies have shown that IGF-1 levels are considered of prognostic value for functional capacity [90, 143]. Additionally, recent studies have associated these entities, as well as the MetS, with low levels of IGF-1, making this hormone a perfect candidate to be considered as a possible treatment.

In order to continue with the new focus of targeting fibrogenesis pathways, to prevent or delay it, the investigation of IGF-1 as a possible therapeutic agent should seriously be taken into account. In the case of NAFLD and NASH, this therapeutic approach reaches a relevant place, as new studies strongly associated these entities, as well as the MetS, with low levels of IGF-1, making this hormone a perfect candidate to be considered as a possible treatment.

Clinical trials to establish the feasible therapeutic doses of IGF-1 in fibrosis and cirrhosis, as well as its specific contribution in each kind of cirrhosis etiology, could be a relevant research target in the next years.

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**Conflicts of Interest**

None.

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**Abbreviations**

ECM: extracellular matrix; GH: growth hormone; GHRH: growth hormone-releasing hormone; HSCs: hepatic stellate cells; IGFBPs: IGF binding proteins; IGFBP-rPs: IGF related proteins; IGF-1: insulin-like growth factor-1; IGF-1R: IGF-1 receptor; MetS: metabolic syndrome; NASH: non-alcoholic steatohepatitis; NAFLD: non-alcoholic fatty liver disease; rhIGF-1: recombinant human insulin-like growth factor-1; rSV40: recombinant simian virus 40; TGF β1: transforming growth factor β1.
growth factor β1

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IGF-I Deficiency and Liver Disease

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