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Revew article

Cu–Zn super oxide dismutase as a potential antifibrotic drug for hepatitis C related fibrosis

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Abstract

Liver fibrosis plays a pivotal role in liver function impairment and is a feature of chronic infection by hepatitis C virus (HCV). Ten to 20% of patients progress to cirrhosis leading to an increased risk of liver failure or hepatocellular carcinoma (HCC). Recent advances have culminated in clear evidence that fibrosis can be reversible, and in expectation that effective anti-fibrotic therapy will improve the prognosis. Among the different cellular pathways involved in fibrosis, the transforming growth factor-β1 (TGF-β1) signaling plays a major role. Increases of TGF-β1 expression is associated with fibrotic diseases and this cytokine could be a target for an antifibrotic drug. Clinical results on radiation-induced fibrosis have brought some evidences that Cu–Zn SOD (SOD1) could be an anti-fibrotic drug. Its therapeutic effect could be related to a down-regulation of TGF-β1 as proved in a well characterized pig model of radiation induced fibrosis, where the efficacy of Cu–Zn SOD has been shown in reversing fibrosis. Using 3-D skin co-culture of fibroblasts from this pig model, it was showed that SOD significantly reduces the expression of the TGFβ1, both at the mRNA and protein level. An experimental work could be undertaken to validate the Cu–Zn SOD as an anti-fibrotic drug, using HCV core protein expressing transgenic mice. As the current anti-HCV therapy with pegylated interferon combined with ribavirin can eradicate virus and stop the progression of fibrosis, but near 50% of HCV infected patients are non responders, a controlled trial is planned for HCV infected patients non responders to this therapy with a Metavir fibrosis score reaching $F = 2$ or > 2.

Keywords: Liver fibrosis; Cu–Zn SOD (SOD1); Stellate cells; TGFβ1; Hepatocellularcarcinoma

1. Introduction

Liver fibrosis plays a pivotal role in liver function impairment [1] and is a common feature of chronic infection by hepatitis C virus (HCV). Ten to 20% of patients progress to cirrhosis [2], increasing the risk of liver failure or hepatocellular carcinoma (HCC) [3]. The rate of fibrosis progression is remarkably variable, and the disease can run from decades of viremia with little fibrosis, to rapid onset of cirrhosis in 10–15 years.

Identified risk factors for rapid progression include: [1]

- Older age at time of infection.
- Alcohol consumption < 50 g/day [4].
- Male gender.
- Increased body mass index associated with hepatic steatosis.
- HIV infection or immunosuppression for liver transplantation.
- Iron overload.

The current anti-HCV therapy with pegylated interferon combined with ribavirin can eradicate virus and stop the progression of fibrosis in HCV patients. Near 50% of HCV infected patients are non responders to this therapy. In such patients fibrosis progress during time, and can lead to cirrhosis [2] increasing the risk of liver failure and (HCC) [3].

Recent advances have culminated in clear evidence that fibrosis and cirrhosis can be reversible, and in realistic expectation that effective anti-fibrotic therapy will significantly improve the prognosis of patient with liver disease [1]

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Among the different cellular pathways involved in fibrosis, the TGF-β1 plays a major role. Increases of TGF-β1 expression associated with fibrotic diseases and this cytokine has been proposed as a target for therapeutic treatments [5,6]. It is interesting to note that in hepatitis C related fibrosis a correlation has been shown between the TGF-β-1 level and the efficacy of the interferon therapy [7].

There is a growing body of evidences that Cu–Zn SOD (SOD1) could be a potential antifibrotic drug, as proved by clinical results on radiation induced fibrosis [8,9]. Its therapeutic effect could be related to a down-regulation of TGF-β1 expression and activity [5,10].

Mechanism of fibrosis and its relationships to inflammation.

Inflammation is a complex tissue response to injury. Persistent injury or inflammatory stimulation, i.e. chronic inflammation, may result in pathological inflammation and pathological fibrotic repair, i.e. fibrosis. Fibrosis may be defined as wounds "where continuous signals for tissue repair are emitted". Such continuous signals arise from stimulating factors like cytokines and growth factors [11,12].

Fibrosis is characterized by massive deposition of extra cellular matrix and excessive fibroblast proliferation. Under normal circumstances once the inflammatory response begins to subside, cytokine regulation of fibroblast activation and proliferation progress to tissue repair, which occurs with minimal scaring. If however repetitive injury occurs like in hepatitis C infection, the tissue cannot heal and overzealous repair evolves to excessive scarring i.e. fibrosis [13].

All these events normal or pathologic are monitored by inflammatory cells, and after the acute phase (where corticosteroids are efficient), especially by monocytes-macrophages, and fibroblasts, which interact and secrete many cytokines/growth factors.

Fibroblasts synthesize collagen and other matrix proteins and these proteins are also being degraded. Both macrophages and fibroblasts are also source of collagenolytic activity. They produce matrix metalloproteinases (MMPs) and collagenases which are crucial enzymes in remodeling normal collagen fabric and scar tissue. Balance between tissue degrading enzymes and inhibition of these enzymes i.e. tissue inhibitor of MMPs (TIMP), both produced by macrophages and fibroblasts is of crucial importance. Excessive fibrotic response is characterized by persistent and excessive presence of myofibroblast, a cell type which is only transiently present during normal wound healing.

Fibroblasts produce many cytokines/growth factors basic fibroblast growth factor (bFGF) transforming growth factor beta (TGF-β), and platelet derived growth factor (PDGF).

TGF-β1 appears to be one of the key cytokine in the cascade of events leading to fibrosis [10,12,20].

Over expressed TGFβ1 simultaneously increases the synthesis of most matrix proteins, decrease the production of matrix-degrading proteases, increases the production of inhibitors of these proteases [1,11].

2. Hepatic fibrosis

In hepatic fibrosis liver macrophages related cells i.e. Kupfer cell, and perivascular stellate cell (previously called. lipocytes, perisinusoidal cell or fat storing cell), play a crucial role [1].

The stellate cells are the key fibrogenic cell. They stay in the subendothelial space of Disse, which separates hepatocytes from sinusoidal endothelium and contains a "basement membrane-like matrix", i.e. extracellular matrix (ECM). This subendothelial matrix contains lattice-like meshwork of ECM molecules, that provide cellular support and allow transport of solutes and growth factors, from sinusoids to hepatocytes. During liver injury the ECM composition become scar like and hepato-cellular functions deteriorate [1].

Activation of stellate cells is the dominant event in fibrogenesis and refers to the conversion of quiescent cells into proliferative, fibrogenic and contractile myofibroblasts. It proceeds along a continuum that involves progressive changes in cellular function [1].

Early events termed initiation renders the cell responsive to host cytokine and stimuli. The perpetuation stage encompasses cellular events that amplify the activated phenotype, resulting in accelerated ECM remodeling i.e. degradation of normal matrix and accumulation of fibrillar matrix.

During resolution of liver fibrosis stellate cells number are diminished by apoptosis, and possibly by reversion to a more quiescent phenotype.

The earliest changes in stellate cells reflect its paracrine stimulation by all neighboring cells type, including sinusoidal endothelium, Kupfer cell, hepatocytes, platelets and leucocytes. This stimulation results in conversion of TGF-β1 from the latent to active profibrogenic form. Le role of Kupfer cell activation and proliferation is prominent. For example TGF-β1 from Kupfer cell markedly stimulate stelatte cell ECM synthesis [14].

TGF-β1 produced by stelatte cells and many others neighboring cells of the liver appears the key cytokine/growth factor involved in liver fibrosis.

In chronic hepatitis C high levels of TGF-β1 had been observed [15] and recent ex vivo and in vitro studies have shown an increase of TGF-β1 synthesis in the presence of HCV proteins [15,16,17]. In these three studies the HCV core protein has been shown to be involved in this increase. Thus in addition to inflammation induced fibrosis HCV may have a direct effect trough induction of TGF-β1.

Oxidative stress and lipid peroxidation have been shown not to be only a feature of the late stage of hepatitis C related fibrosis [18]. Activated neutrophils, Kupfer cells, platelets generate reactive oxygen species (ROS), especially O_2^- [19]. They are able to stimulate collagen synthesis in hepatic stellate cells, as ROS derived from P450 2EI [20]. Thus the involvement of oxidative stress in hepatic fibrogenesis is clearly demonstrated [21,22], notably by the up regulation of TGF-β1 expression from 4-hydroxynonenal [23] and by inhibition of gene expression of TGF-β1 in rats with vitamin E supplementation [24].