Adipokines and liver fibrosis

F. MARRA¹,², S. ALEFFI¹, C. BERTOLANI¹, I. PETRAI¹, F. VIZZUTTI¹

¹Dipartimento di Medicina Interna and ²Center for Research, Transfer, and High Education “MCIDNENT”, University of Florence, [Italy]

Abstract. – Liver fibrosis involves different cell types, and should be regarded as a “wound healing” response that occurs in conditions of chronic liver injury and is characterized by inflammation, activation of matrix-producing cells, matrix deposition and remodeling, and epithelial cell regeneration or an attempt thereof. Liver damage may be caused by several agents or conditions, resulting in different degrees and types of tissue inflammation and in activation of matrix-producing cells, such as the hepatic stellate cells (HSC). HSC undergo a phenotypic transition (known as “activation”) to myofibroblast-like cells that synthesize different extracellular matrix components. Obesity is associated with the development of NASH, and has been indicated as an independent factor for the progression to fibrosis. In liver diseases, the biologic actions of the adipokines, such as leptin, adiponectin and resistin, released by adipocytes or locally produced by liver and/or inflammatory cells, may contribute to clarify the mechanisms of progression in NASH. The clinical and experimental findings accumulating on this class of molecules could represent the basis to devise a better management strategy for the patients with chronic liver disease.

Key Words:
NASH, Adipokines, Fibrogenesis, Hepatic stellate cells.

General Concepts on Liver Fibrogenesis

Liver fibrosis is a highly coordinated process involving different cell types, and should be regarded as a “wound healing” response occurring in conditions of chronic liver injury. The basic elements of wound healing in any tissue are represented by damage, inflammation, activation of matrix-producing cells, matrix deposition and remodeling, and finally epithelial cell regeneration or an attempt thereof. Liver damage may be caused by a number of agents or conditions, resulting in different degrees and types of tissue inflammation and in activation of matrix-producing cells, a pivotal event in the pathway leading to fibrosis. The best known cellular elements responsible for the development of fibrosis are the hepatic stellate cells (HSC)¹. HSC undergo a phenotypic transition (known as “activation”) to myofibroblast-like cells that synthesize different extracellular matrix components, including fibrillar collagens, and fibronectin. Activated HSC are highly proliferative and migrate promptly in response to chemotactic agents, resulting in spatial and quantitative differences with respect to the deposition of extracellular matrix in different types of injury. In addition, HSC are responsible for the secretion of many soluble factors, including cytokines, that modulate recruitment of inflammatory cells and of additional HSC, thus amplifying the inflammatory and fibrotic process. Thus, HSC are regarded to as coordinators of the wound healing response within the liver, due to their multifaceted actions in the processes taking place during tissue damage. It should be pointed out that analysis of different cellular markers in pathologic specimens from human liver diseases and rodent models of fibrosis indicates that HSC are not the only cellular contributors to the deposition of extracellular matrix during chronic liver diseases, and that the relative role of different cell types depends on the cause of fibrosis². Because HSC are localized within the hepatic lobule, they are most likely to play a pivotal role in conditions where deposition of matrix occurs around the sinusoids in a pericellular or “chicken-wire” fashion, as observed in alcoholic or nonalcoholic steatohepatitis. HSC are also involved in the processes leading to
tissue remodeling and hepatocyte regeneration. In fact, once the injuring stimulus is removed, reversal of fibrosis, at least in rodents, is preceded by apoptosis of HSC. These findings suggest that promotion of HSC apoptosis may be a possible tool to reduce tissue scarring. Finally, HSC secrete soluble factors that regulate hepatocyte survival and proliferation, such as hepatocyte growth factor, and their production is modulated during the process of HSC activation.

Mechanisms of Fibrosis During Nonalcoholic Steatohepatitis

Only a limited amount of information is currently available on the possible role of pro-and anti-fibrogenic factors during nonalcoholic steatohepatitis (NASH). It is well established that in conditions of insulin resistance the liver accumulates triglycerides, resulting in the developments of steatosis. Steatosis is a condition necessary but not sufficient for the development of steatohepatitis and fibrosis, that according to the “two-hit” hypothesis require an additional “hit”. Nevertheless, studies conducted in large series of well-characterized NASH patients have indicated that the severity of insulin resistance is independently associated with the degree of fibrosis in the liver biopsy. These data support the possibility that insulin resistance constitutes also the “second hit” (or part thereof) leading to steatohepatitis and fibrosis. Some mechanistic evidence supporting this possibility has been obtained in cultured HSC, where insulin increases cell proliferation and collagen production, and up-regulates the expression of pro-fibrogenic cytokines such as connective tissue growth factor.

Generation of oxidative stress-related molecules, such as reactive oxygen intermediates and reactive aldehydes, has been suggested as a critical factor responsible for the progression from simple steatosis to NASH and fibrosis. Compelling evidence indicates the existence of oxidative stress in the liver of patients with NASH or of animals with experimentally induced NASH. Moreover, oxidative stress is implicated in the activation of quiescent HSC, and both oxidant and non-oxidant products of oxidative stress exert pro-fibrogenic actions on culture-activated HSC. Thus, production of hydrogen peroxide is implicated in the expression of procollagen type I, and reactive oxygen intermediates generated inside the cells participate in the signal transduction of several cytokines which up-regulate extracellular matrix components. Reactive aldehydes, including 4-hydroxy-nonenal, a non-oxidant product of lipid peroxidation, increases procollagen expression in cultured human HSC, and upregulates pro-inflammatory cytokines such as MCP-1. Finally, interfering with the processes of lipid peroxidation is effective in reducing fibrosis in experimental models.

The possible role of inflammation in the development of fibrosis during NASH is more controversial. In chronic viral hepatitis, moderate-severe inflammation is associated with faster progression of fibrosis, but only minimal inflammation is present in other chronic diseases characterized by progression to cirrhosis. Although inflammation is a common histological finding in patients with NAFLD, its presence is not sufficient for the diagnosis of NASH in the absence of hepatocellular damage and/or fibrosis. Matteoni et al. have reported that the presence of fat and inflammation alone is associated with a low likelihood to progress to cirrhosis, although the number of patients in this group was somehow limited. On the other hand, several studies have found that necro-inflammation, or ALT levels correlate with the presence or degree of fibrosis. Data obtained in cultured HSC and in other matrix-producing cells, indicate that inflammation and fibrosis are closely linked. First, molecules produced by cells of the inflammatory infiltrate, such as platelet-derived growth factor, are potent stimulators of fibrogenesis. Conversely, some of the molecular mechanism responsible for the induction of inflammation are also able to promote fibrosis. The chemokines represent a typical model system connecting the recruitment of inflammatory cell to the modulation of HSC's biology in a pro-fibrogenic manner, and may provide an explanation for the close spatial relationship between matrix accumulation and inflammation observed in some conditions, such as viral hepatitis. Despite these lines of evidence suggesting the contribution of inflam-
information in the development of fibrosis, mechanistic studies investigating this aspect of the pathogenesis of steatohepatitis are needed.

**Adipokines**

The growing attention of the scientific community towards NASH as one of the causes of end-stage liver disease is spurred by the rising prevalence of risk factors for nonalcoholic steatosis in the western population. Obesity is a well recognized condition associated with the development of NASH, and in some series it has been indicated as an independent factor for the progression to fibrosis. Once merely considered as a deposit of excess energy in the form of triglycerides, it is now accepted that adipose tissue is a store of bioactive molecules that act locally, as cytokines, or at distance, in an endocrine fashion. For these reasons, the group of cytokines secreted by the adipocytes, collectively known as adipokines, represent an area of active research not only in the field of NASH but in all other conditions associated with obesity and the metabolic syndrome. It should be underscored that adipose tissue is a source of factors that exert potent inflammatory actions on liver cells. These include cytokines such as TNF-α, interleukin-6, or the chemokine MCP-1, and molecules involved in matrix turnover, including plasminogen-activator inhibitor-1.

Leptin is a prototypical adipokine, which has been identified for its ability to control energy homeostasis acting on the hypothalamus, where it regulates body weight homeostasis and negative feedback. In rodents, obesity usually associates with elevated leptin levels due to increased fat mass. Originally thought to be only a satiety factor, leptin is a pleiotropic molecule, playing important roles in immune function, fertility, bone formation and wound healing, and is produced by many tissues besides the fat. The influence of leptin on the pathophysiology of different systems also extends to the liver, where leptin modulates the response to different types of injury. Leptin deficiency is associated with increased hepatotoxicity and mortality following endotoxin administration, and with reduced liver damage in models of T cell-mediated hepatitis. A first clue indicating a connection between leptin and liver fibrosis dates back to 1998 when Potter et al. reported that activated, but not quiescent, HSC express leptin. The initial observation of leptin production by HSC has been followed more recently by a number of in vivo and in vitro studies showing that leptin has a profibrogenic action on the liver, related, at least in part, to a direct effect on the biology of matrix-producing cells, such as the hepatic stellate cells (HSC). Injection of exogenous leptin to rats intoxicated with thioacetamide leads to more severe hepatic damage, indicated by higher ALT levels, and fibrogenesis. On the other hand, the absence of leptin or leptin signaling results in a marked reduction of liver fibrosis induced by different conditions, including thioacetamide intoxication, chronic CCl₄ administration or experimental NASH.

Despite the general agreement that leptin positively regulates fibrogenesis, the molecular and cellular mechanisms underlying this effect are still debated. At least 6 isoforms of leptin receptors (ObR) are generated by alternative splicing. The “long” form of the receptor, ObRb is capable of fully transducing leptin’s signals, while the short forms, particularly the widely expressed ObRa, possess limited signaling capabilities. Using RT-PCR, Ikejima et al. were unable to detect transcripts for ObRb in activated rat HSC, and suggested that the major effect on collagen expression depends on an autocrine loop involving secretion of TGF-β by endothelial cells, which express ObRb. On the other hand, Saxena et al. demonstrated a direct fibrogenic action on primary and immortalized rat cells, and found detectable ObRb expression at the gene and protein levels, together with activation of Stat3, a signaling pathway that is unique to the “long” receptor (Ahima 2004). Similar discrepancies have been reported in immortalized human HSC lines, where one group found only the short isoform of the leptin receptor, while others showed expression of both the long and the short forms of ObR.
Studies conducted on cultured cells demonstrate that leptin exerts a number of biological actions on HSC, including up-regulation of collagen, stimulation of cell proliferation and prevention of apoptosis\(^2\). Preliminary data from our group also indicate that leptin stimulates secretion of pro-inflammatory and pro-angiogenic cytokines by cultured human HSC\(^3\). Despite these compelling data, clinical studies have failed to clearly demonstrate an association between plasma leptin levels and the degree of fibrosis in patients with NASH, although this correlation has been recently shown in patients with chronic HCV-related hepatitis\(^4\). It is possible that intra-hepatic, rather than circulating leptin contributes its pro-fibrogenic action through autocrine and paracrine actions, in a cytokine-like fashion. Future studies should be designed to better understand the relative contribution of circulating vs. locally produced leptin in the regulation of liver response to injury.

Adiponectin is a recently identified protein that is predominantly, but not exclusively, expressed by the adipose tissue. It circulates at high levels in the bloodstream, representing one of the main plasma proteins. Interestingly, adiponectin is present in both a full length, high molecular weight form and in a truncated, globular form\(^4\), the biologic significance and spectrum of action of which is still under investigation. Adiponectin is considered a major determinant of the sensitivity to insulin, acting at different sites of glucose metabolism, including liver, muscle, and fat itself\(^4\). Adiponectin levels have been found to be decreased in patients with obesity and/or type II diabetes, suggesting a possible role in the development of the phenotype of the metabolic syndrome. For these reasons, there is growing attention on the role played by this molecule in patients with NASH. Recent data indicate that levels of adiponectin are more markedly reduced in patients with NASH than in those with simple steatosis\(^4\), suggesting that measurement of plasma levels of this hormone may contribute to diagnose the more severe forms of NAFLD. In addition, experimental data also show that administration of recombinant adiponectin ameliorates metabolic derangements and liver damage in mouse models of alcoholic and nonalcoholic steatohepatitis\(^4\). Thus, adiponectin may block the development of fibrosis limiting hepatic damage. More recently, a direct antifibrogenic action of adiponectin has been demonstrated in animals undergoing toxic liver damage\(^4\), a condition independent of deranged metabolism. Lack of this hormone in adiponectin-deficient mice was associated with a more marked induction of fibrosis upon chronic administration of carbon tetra-chloride. Conversely, injection of adeno-virus producing adiponectin before carbon tetra-chloride or 6 weeks after the induction of damage attenuated liver fibrosis. Adiponectin's effects are mediated by two receptors, known as AdipoR1 and AdipoR2\(^4\), and at least some of the metabolic effect of adiponectin are dependent on receptor-mediated activation of AMP-dependent protein kinase\(^4\). However, the contribution of the different receptor isoforms and/or AMP-dependent kinase to the antifibrogenic effects of adiponectin has not yet been elucidated. The emerging biology of adiponectin make this molecule a very appealing target for future studies in NASH and other liver diseases.

Other adipokines are possibly implicated in the fibrogenic process. Resistin contributes to insulin resistance in rodents, but its metabolic effects in humans are still uncertain\(^4\). Preliminary evidence obtained in our laboratory indicates that resistin modulates the biology of human HSC inducing a pro-inflammatory phenotype. In addition, like reported for other “adipokines”, the expression of resistin is detectable in liver tissue, especially in conditions of fibrosis\(^4\). Remarkably, recently published data show that resistin also induces an increase in the growth of vascular smooth muscle cells through a ERK-dependent pathway\(^5\).

Conclusions

The adipokines represent a novel and intriguing area of investigation in many fields of medicine. In liver diseases, the biologic actions of these molecules released by adipocytes or locally produced by liver and/or inflammatory cells, may contribute to reveal the mechanisms of progression in nonalcoholic steatohepatitis. However, the interest of
these molecules extends beyond NAFLD, because obesity is a well established risk factor for the progression of fibrosis also in chronic viral hepatitis. The clinical and experimental findings accumulating on this class of molecules will likely represent the basis to devise a better management strategy for the patients with chronic liver disease.

References

1) Reeves HL, Friedman SL. Activation of hepatic stellate cells—a key issue in liver fibrosis. Front Biosci 2002; 7: d808-826.


Acknowledgements

Work in Dr. Marra’s laboratory is supported by grants from MIUR (PRIN 2002, PRIN 2004, FIRB), from the University of Florence, and from the Italian Liver Foundation.