Role of hepatic stellate cells in the pathogenesis of portal hypertension

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BACKGROUND

Hepatic stellate cells (HSC) are located in the space of Disse in close contact with hepatocytes and sinusoidal endothelial cells. In human liver, HSC are located along the sinusoids with a nucleus-to-nucleus distance of 40 µm, indicating that the sinusoids are equipped with HSC at certain fixed distances. These observations suggest that, although the total number of HSC constitutes a small percentage of the total number of liver cells (approximately 5-8%), their spatial disposition and spatial extension may be sufficient to cover the entire hepatic sinusoidal microcirculatory network. The most conspicuous ultrastructural feature of HSC in normal adult liver is the presence of cytoplasmic lipid droplets ranging in diameter from 1-2 µm (i.e., «fat-storing cells» or «lipocytes»). These lipid droplets are important in the hepatic storage of retinyl esters, and, accordingly HSC have been shown to play a key role in the metabolism of retinoids.

The role of HSC in the progression of liver fibrosis has been extensively characterized. As a consequence of chronic liver tissue damage, HSC, as well as other extracellular matrix-producing cells (e.g. fibroblasts and myofibroblasts constitutively present in the portal tract), undergo a process of activation that leads to a phenotype characterized by increased proliferative, motile and contractile properties.

The recognition that HSC have contractile properties represents a key acquisition in the knowledge of the biology of this cell type (see for review). Contraction of activated HSC occurs in vitro in response to different vasoconstrictors (table I). However, these findings are likely to be more representative of HSC contractile status in fibrotic liver, where contraction of activated HSC in response to various stimuli may have important implications in the pathogenesis of portal hypertension and in the contraction of mature scar tissue. Following two studies published in 1992 demonstrating the contraction of HSC in response to various vasoconstrictors, the potential involvement of this cell type in the genesis and progression of portal hypertension has reached a level of potential misunderstanding. For this reason, it is necessary to review this issue and reach clear-cut conclusions.

In order to proceed in a rational fashion, this paper will address three specific issues: 1. Do HSC play a role in the regulation of sinusoidal tone in the normal liver? 2. Do HSC influence portal pressure in conditions of developing fibrosis and «capillarization» of sinusoids? 3. Do HSC influence portal pressure in the cirrhotic liver?

POTENTIAL ROLE OF STELLATE CELLS IN THE REGULATION OF SINUSOIDAL TONE IN NORMAL LIVER

Because of their anatomical location, ultrastructural features, and similarities with pericytes regulating blood flow in other organs, HSC have been proposed to function as liver-specific pericytes. Branches of the autonomic nerve fibers coursing through the space of Disse come in contact with HSC, and nerve endings containing substance P and vasoactive intestinal peptide have been demonstrated in the vicinity of HSC.

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Table I. Action of vasoactive agents on hepatic stellate cells

<table>
<thead>
<tr>
<th>Agent</th>
<th>Contraction</th>
<th>Relaxation</th>
<th>[Ca^{2+}] increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endothelin-1</td>
<td>++++</td>
<td></td>
<td>Coupled</td>
</tr>
<tr>
<td>Thrombin</td>
<td>++++</td>
<td></td>
<td>Coupled</td>
</tr>
<tr>
<td>Angiotensin-II</td>
<td>+++</td>
<td></td>
<td>Coupled</td>
</tr>
<tr>
<td>Substance P</td>
<td>+++</td>
<td></td>
<td>Coupled</td>
</tr>
<tr>
<td>Adenosine</td>
<td>+++</td>
<td></td>
<td>Coupled</td>
</tr>
<tr>
<td>Thromboxane</td>
<td>+++</td>
<td></td>
<td>Coupled</td>
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<tr>
<td>Vasopressin</td>
<td>++++</td>
<td></td>
<td>Coupled</td>
</tr>
<tr>
<td>Platelet-activating factor</td>
<td>+</td>
<td></td>
<td>Coupled</td>
</tr>
<tr>
<td>Cysteinyi Leukotrienes</td>
<td>+++</td>
<td></td>
<td>Coupled</td>
</tr>
<tr>
<td>Adrenomedullin</td>
<td>++</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>Nitric Oxide</td>
<td>++</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>cAMP increasing agents</td>
<td>+++</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>Lipo PGE</td>
<td>++</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atrial Natriuretic Peptide</td>
<td>+++</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>C-type Natriuretic Peptide</td>
<td>+++</td>
<td>*</td>
<td></td>
</tr>
</tbody>
</table>

*: relaxation associated with an inhibition of vasoconstrictor induced-[Ca^{2+}] increase
In both normal and fibrotic liver, the expression of N-CAM, a typical central nervous system adhesion molecule detected in hepatic nerves, and the expression of glial fibrillary acidic protein (GFAP) are restricted, among liver cell types, to HSC. These observations, while reinforing a potential functional relationship between the autonomic nervous system and HSC, raise a current key issue concerning the origin of this cell type, previously considered to be of myogenic origin in reason of the expression of desmin and smooth muscle α-actin (α-SMA). Along these lines, activated HSC express nestin, a class VI intermediate filament protein originally identified as a marker for neural stem cells. Remarkably, the expression of this cell marker appears to be restricted to HSC and pericytes of brain parenchyma vessels, among all organ-specific pericytes. Another neuroendocrine marker suggesting a combination of mesenchymal and neural/neuroendocrine features in HSC is synaptophysin, a protein involved in neurotransmitter exocytosis. Synaptophysin reactivity is present in perisinusoidal stellate cells in both human and rat normal liver biopsies and the number of synaptophysin-reactive perisinusoidal cells is increased in pathological conditions. Recent experimental evidence indicate that rat and human HSC express neurotrophins (including nerve growth factor -NGF-, brain-derived neurotrophin, neurotrophin 3 and neurotrophin 4/5) and neurotrophin receptors. This information does not support the possible neural/neuroendocrine differentiation of HSC since neurotrophins and the relative receptors have been identified in a variety of mesenchymal cells, such as fibroblasts and myofibroblasts, both in normal tissues and in tissues undergoing acute or chronic wound repair. Expression of neurotrophins in tissues other than the central or peripheral nervous system has been classically considered to be aimed at stimulation of outgrowth and maintenance of the peripheral nervous system. However, an increasing number of experimental reports indicate that the neurotrophin/neurotrophin receptor systems is likely implicated in biological events such as cell differentiation, proliferation, survival and motility. In addition, a significant positive correlation has been reported in vascular smooth muscle cells between NGF synthesis and cell contractility, possibly related to the regulation of intracellular calcium homeostasis. In aggregate, these observations suggest a complex interaction between the pathophysiological role of HSC and the function of the peripheral nervous system. Accordingly, further studies aimed at clarifying the embryonic origin of this cell type and the biological purposes of this interaction are strongly encouraged.

Although evidence suggests a role of HSC in the regulation of sinusoidal blood flow in normal liver, this issue is still controversial. In a review article published in 1999, Etakaksin and Kaneda raised several issues, mostly from the anatomical standpoint, arguing against the role of HSC in the regulation of sinusoidal blood flow. When visualized in three dimensions, HSC do not have a stellate form (typical of their aspect in bidimensional culture on plastic) but rather a «spider-like» appearance («arachnocytes») consisting of a small cell body with a series of radiating and parallel slender processes. According to these authors, cells with this tridimensional feature are not likely to be «contraction ready». Additional limitations to effective cell contraction are offered by the spatial limitation of the space of Disse, by the intracytoplasmic presence of lipid droplets that prevent microfilaments from assembly in a longitudinal span, and by the ultrastructural evidence of limited development of contractile filaments in quiescent HSC. Regardless, studies evaluating the hepatic microcirculation by intravital microscopy techniques have suggested that HSC could be involved in the regulation of sinusoidal tone in normal liver. An additional matter of debate is provided by studies aimed at quantitating HSC contraction with techniques able to detect the development of contractile forces in response to vasoconstrictors. The results of these studies indicate that the magnitude and kinetics of contraction and relaxation are consistent with the hypothesis that HSC may affect sinusoidal resistance. However, for understandable technical reasons, these data were obtained in rat HSC in primary culture 7 days after isolation, when a certain degree of activation in culture has occurred. In conclusion, although HSC could be proposed as liver-specific pericytes because of their location, spatial distribution, relationship with the peripheral nervous system, and ultrastructural features, no conclusive evidence is presently available concerning their role in the regulation of sinusoidal blood flow under physiological conditions. Hopefully, further technical improvements would be helpful in resolving this issue in the near future.

DO STELLATE CELLS INFLUENCE PORTAL PRESSURE IN DEVELOPING FIBROSIS AND «CAPILLARIZATION» OF SINUSOIDS?

As stated previously, a remarkable increase in HSC contractile properties is likely a key feature of their activated state. At this stage, HSC have been shown to express a large number of voltage-operated calcium channels, the activation of which is associated with an increased intracellular calcium concentration followed by marked cell contraction.
These changes are possibly dependent on intracellular and extracellular factors. First, the transition to the «myofibroblast-like» phenotype is ultrastructurally characterized by the appearance of massive contractile structures including dense bodies and patches of myofilaments throughout the cytoplasm. Second, HSC activation is accompanied by increased expression of α-SMA, and it is likely that this cytoskeletal protein is, at least in part, responsible for increased cell contractility. Interestingly, both pro-fibrogenic agents and vasoconstrictors represent potential regulators of the α-SMA gene, and, in this context, the transcription factor c-myb has been shown to form complexes with a regulatory element of the α-SMA gene, suggesting that induction of this gene may be transcriptionally regulated. Among «external» factors that could affect HSC contractility, the modified ECM pattern typical of fibrotic liver is also likely to play an important role. The presence of a microenvironment rich in fibrillar ECM and the expression of integrin receptors specific for the constitutive components of this ECM (particularly collagen type I and III) lead to a structural configuration of activated HSC characterized by cytoskeletal tension or stress. This feature of activated HSC is likely to be relevant for the modulation of different cell functions including proliferation and migration in response to growth factors and contraction in response to vasoconstrictors. It is indeed logical to hypothesize that HSC contractile status could be conditioned by the presence of vasoactive substances present in the microenvironment of hepatic tissue undergoing active fibrogenesis.

As a consequence of their activated state, HSC contribute to profound alteration of the sinusoidal structure during the early stages of hepatic fibrogenesis. Capillarized sinusoids are characterized by accumulation of fibrillar extracellular matrix in the space of Disse. In this context, endothelial cells are characterized by a loss of their fenestrations thus acquiring a «generic» endothelial cell phenotype (denoted by the positivity for factor VIII). These changes are associated with: a) impairment in the metabolic exchange between blood and hepatocytes, b) impairment in the natural dispersion of hydrostatic forces that occurs in the normal sinusoidal sieve. Capillarization of sinusoids is likely to represent an initial cause of portal hypertension during the early development of hepatic fibrosis. In conditions characterized by portal tract expansion and periportal fibrosis, such as chronic viral hepatitis and primary biliary cirrhosis, HSC activation occurs in periportal sinusoids, thus contributing to the so-called «early pre-sinusoidal resistance locus». In other conditions, such as chronic alcoholic hepatitis and non-alcoholic steatohepatitis, capillarization of sinusoids is initially limited to the center of the liver lobe, around the centrilobular vein, resulting in obstruction to sinusoidal blood flow.

It is likely that activated HSC, together with other contractile cells present in the liver tissue, play an important role in the development of portal hypertension only during initial fibrotic transformation. However, at these early stages in the fibrogenic evolution of chronic liver diseases portal hypertension is not yet clinically relevant. This is likely due to the substantial integrity of hepatic angioarchitecture and to the compensatory adjustments occurring at the presinusoidal level in the intrahepatic portal circulation.

DO STELLATE CELLS INFLUENCE PORTAL PRESSURE IN CIRRHOTIC LIVER?

The hallmark of any form of cirrhosis is a profound alteration of the liver angioarchitecture with two prominent features: a) development of septal fibrosis establishing portal-central anastomoses, and b) arterialization and capillarization of sinusoids due to both reduction of portal flow and formation of «feeding vessels» derived from the hepatic artery. These changes per se could be sufficient to explain the increase in portal pressure typical of liver cirrhosis. Indeed, portal-central anastomoses, although representing direct connections between the portal and the systemic circulation, follow irregular patterns and are embedded in a developing scar tissue undergoing, to a certain extent, spontaneous retraction. In addition, these neformed vessels could be the site of thrombosis, thus aggravating the intrahepatic hemodynamic disturbances. These general alterations are typical of postnecrotic cirrhosis. In other types of cirrhosis, additional factors may play a role. In alcoholic cirrhosis, compression of hepatic venules by scar tissue that develops around the central vein (pericentral fibrosis) and marked hepatocellular swelling may aggravate portal hypertension. In primary or secondary biliary cirrhosis, distortion of portal vein branches secondary to a progressive portal-portal fibrosis, may represent a «presinusoidal» cause of portal hypertension.

It is very clear that all these potential causes of portal hypertension are likely irreversible and not likely affected by pharmacologic intervention. Particularly in the case of septal fibrosis, the establishment of portal-central anastomoses likely represents a «point of no-return» for the fibrogenic process as the profound derangement of hepatic angioarchitecture causes additional liver tissue damage perpetuating and aggravating the fibroproliferative process. However, this view bears the same defect of the clas-
Role of Hepatic Stellate Cells in the Pathogenesis of Portal Hypertension

**Vasoactive Agents Affecting HSC Biology and Their Potential Role in Portal Hypertension**

Several vasoactive agents have been shown to be effective in modulating activated HSC contractility in culture (Table I). The role of two vasoregulatory compounds, namely endothelin 1 (ET-1) and nitric oxide (NO), has been particularly highlighted.

Endothelin-1, a potent vasoactive 21-amino-acid peptide secreted by endothelial as well as other cell types, has been shown to exert a multifunctional role in a variety of tissues and cells, including the liver. Infusion of ET-1 in the isolated perfused rat liver causes a sustained and dose-dependent increase in portal pressure associated with increased glycogenolysis and oxygen consumption. ET-1 stimulates glycogenolysis, phosphoinositide turnover and repetitive, sustained intracellular calcium transients in isolated rat hepatocytes. Other studies indicate that ET-1 may also have important interactions with liver nonparenchymal cells. Cultured sinusoidal endothelial cells isolated from rat liver have been shown to release ET-1, and preferential binding sites for ET-1 have been identified, both in vivo and in vitro, on HSC. As previously mentioned, ET-1 induces a dose-dependent increase in intracellular free calcium, coupled with cell contraction in this cell type. Importantly activated rat and human HSC have been shown to express preproET-1 mRNA and to release ET-1 in cell supernatants in response to agonists such as angiotensin II, PDGF, TGF-β and ET-1 itself, thus raising the possibility of a paracrine and autocrine action of ET-1. Recent data indicate that ET-induced ET-1 synthesis in HSC is regulated through modulation of endothelin converting enzyme-1 (ECE-1), rather than by modulation of the precursor pre-proET-1. Overall, it is increasingly evident that the process of HSC activation and phenotypical modulation is characterized by a close and complex relationship with the ET system. The ability to synthesize and release ET-1 is associated with a progressive shift in the relative predominance of ET_α_ and ET_β_ receptors observed during serial subculture: ET_α_ are predominant in the early phases of activation, whereas ET_β_ become increasingly more abundant in «myofibroblast-like» cells. This shift in the relative receptor densities may be directed at differentiating the possible paracrine and autocrine effects of ET-1 on HSC during the activation process. Indeed, when HSC are provided with a majority of ET_β_ receptors (early phases of activation), stimulation with ET-1 causes a dose-dependent increase in cell growth, ERK activity and expression of c-fos. These effects, likely related to the activation of the Ras-ERK pathway, are completely blocked by pre-treatment with BQ-123, a specific ET_α_ receptor antagonist, and are in agreement with studies performed in other vascular pericytes such as glomerular mesangial cells. In later stages of activation, when the number of ET_β_ receptors increases, ET-1 appears to induce a prevalent antiproliferative effect linked to the activation of this receptor subtype. In this setting the activation of the ET_β_ receptor stimulates the production of prostaglandins, leading to an increase in intracellular cAMP, which in turn reduces the activation of both ERK and JNK. In addition, both cAMP and prostaglandins upregulate ET_β_ binding sites, thus suggesting the possibility of a positive feedback regulatory loop. In this context it is important to note that, at least in human HSC, ET-1-induced cell contraction occurs at any stage of HSC activation. Since HSC contraction is always blocked by ET_α_ receptor antagonists and never reproduced by selective ET_β_ agonists, it is conceivable that the signaling pathways regulating HSC contraction require the activation of a small number of ET_α_ receptors and are somehow divergent from those regulating cell growth.
In aggregate these observations suggest that ET-1 may act as a potent vasoconstrictor agonist regulating intrahepatic blood flow in cirrhotic liver with a potential role in the pathogenesis of portal hypertension. Along these lines, morphological studies have clearly indicated that ET-1 (both at mRNA and protein levels) is markedly overexpressed in different cellular elements present within cirrhotic liver tissue, and particularly in sinusoidal endothelial and HSC in their activated phenotype located in the sinusoids of the regenerating nodules, at the edges of fibrous septa, and in the ECM embedding neoformed vessels within fibrous bands. In addition, clinical studies indicate that a direct relationship exists between ET receptor mRNA abundance and the degree of portal hypertension in cirrhotic patients.

Nitric oxide is a small, relatively stable, free-radical gas that readily diffuses into cells and membranes where it reacts with molecular targets. Importantly, the precise biochemical reactions, which are realized in any biological setting, depend on the concentration of NO achieved and often on subtle variations in the composition of the intra- and extracellular milieu. Accordingly, the biological actions of NO are often defined as a «double-edged sword». NO may act as a key signaling molecule in physiological processes as diverse as host-defense, neuronal communication, and regulation of vascular tone. On the other hand, excessive or not adequately regulated NO synthesis has been implicated as causal or contributing to several pathophysiological conditions including vascular shock, diabetes, and chronic inflammation. Although, NO is characterized by a very short half-life, its biochemical interactions with oxyradicals lead to the production of longer-lived compounds such as peroxynitrite, with important local effects. NO is produced from L-arginine by one of the three isoforms of nitric oxide synthase (NOS). The «constitutive» forms of NOS, that respond to changes in intracellular calcium concentration and typically produce small amounts of NO, are expressed by endothelial cells and in neurons, whereas a wide variety of other cells express the «inducible» form of this enzyme, that binds calmodulin at virtually all calcium concentrations and produce remarkably higher amounts of NO. The constitutive forms are regulated by hypoxia, stretch or cytokines, whereas the inducible form is regulated by a large variety of stimuli including cytokines and lipopolysaccharide. Because of the complex regulation of NOS expression and activity and of the diverse and often opposite effects of NO, the role of this system in several disease states is still largely conjectural, and this applies to liver physiology and pathology as well.

Since the intraportal administration of the NOS inhibitor, Nω-nitro-L-arginine, increases portal pressure, NO has been postulated to be a regulator of sinusoidal blood flow in normal liver. Along these lines, in vitro and in vivo evidence indicates that sinusoidal endothelial cells express constitutive nitric oxide synthase (eNOS) and produce NO, and increase their production in response to flow. However, endothelial dysfunction associated with a decreased production of NO in the intrahepatic microcirculation has been extensively documented in cirrhotic liver, and could directly contribute to the increased intrahepatic resistance typical of portal hypertension. This view is supported by experiments performed in vitro and in animal models by gene transfer of the neuronal NO synthase isoform (nNOS) to sinusoidal endothelial cells or other perisinusoidal cells, such as HSC. Expression of nNOS in rat HSC and sinusoidal endothelial cells resulted in increased NO production, and, in HSC, in a reduction of ET-1-induced contractility. Moreover, in two different rat models of cirrhosis and portal hypertension, transduction of livers with recombinant Ad.nNOS significantly reduced intrahepatic resistance and portal pressure.

In the case of ET-1, in vitro studies have provided circumstantial evidence for a relevance of NO in HSC biology. Exogenous NO is able to prevent ET-1 induced contraction and relax precontracted cells, and also reduces the expression of α-SMA. In addition, interferon-γ and other cytokines with or without lipopolysaccharide, as well as hyaluronan fragments induce the expression of the inducible form of NOS and the production of NO in HSC. However, at least in human HSC, this effect is very limited and the possibility of an autocrine action of NO in HSC appears merely speculative. In addition to these effects on HSC contraction and contractile proteins, NO has been shown to reduce the expression of procollagen type I mRNA and the secretion of the encoded protein. It is therefore possible that NO may influence the progression of portal hypertension by reducing the accumulation of fibrillar matrix in key areas such as the fibrous septa, as suggested by animal models of liver fibrosis. It is also conceivable that the reduced synthesis of NO, typical of cirrhotic liver, may further aggravate the fibrogenic progression of the disease and that administration of orally active NO-donors could be proposed as a potential antifibrogenic treatment, as suggested by recent studies performed in human HSC.

Other studies have evaluated the effects of naturally occurring vasodilators on HSC contractility. These include atrial natriuretic peptide (ANP) and C-type natriuretic peptide (CNP). Both these agent...
have been shown to reduce HSC contraction in response by ET-1 or thrombin. In addition CNP is able to reduce HSC proliferation induced by PDGF-BB. Although increased circulating levels of ANP and other natriuretic peptides have been reported in patients with decompensated cirrhosis, no definitive information is available concerning their possible action within cirrhotic liver tissue.

In addition to ET-1, the potential involvement of other vasoconstrictors synthesized and released within liver tissue has been suggested. Titos and coworkers have recently reported that in cirrhotic rat liver there is an increased synthesis of cysteinyl leukotrienes (LTs). In this context, hepatocytes exhibit the highest ability to generate cysteinyl-LTs. Importantly these compounds elicit a strong contractile response by ET-1 or thrombin. In addition CNP is able to reduce HSC proliferation induced by PDGF-BB. This finding further reinforces the concept of an imbalance between vasoconstrictor and vasodilator agents within the intrahepatic circulation of cirrhotic liver. Importantly, the concentration of vasoconstrictors acting on the intrahepatic microvasculature of cirrhotic liver may increase as a consequence of clinical or subclinical events such as infections in the peritoneal cavity, which are clearly associated with a worsening of portal hypertension and with an increased incidence of variceal bleeding. Appropriate use of drugs currently indicated for the treatment of portal hypertension should be carefully re-considered in light of the current knowledge of the cellular and molecular mechanisms of portal hypertension.

REFERENCES