TOPIC HIGHLIGHT

Thrombin activation and liver inflammation in advanced hepatitis C virus infection

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Abstract

Hepatitis C virus (HCV) infection is associated with increased thrombotic risk. Several mechanisms are involved including direct endothelial damage by the HCV virus, with activation of tissue factor, altered fibrinolysis and increased platelet aggregation and activation. In advanced stages, chronic HCV infection may evolve to liver cirrhosis, a condition in which alterations in the portal microcirculation may also ultimately lead to thrombin activation, platelet aggregation, and clot formation. Therefore in advanced HCV liver disease there is an increased prevalence of thrombotic phenomena in portal vein radicles. Increased thrombin formation may activate hepatic stellate cells and promote liver fibrosis. In addition, ischemic changes derived from vascular occlusion by microthrombi favor the so called parenchymal extinction, a process that promotes collapse of hepatocytes and the formation of gross fibrous tracts. These reasons may explain why advanced HCV infection may evolve more rapidly to end-stage liver disease than other forms of cirrhosis.

Key words: Coagulation; Liver cirrhosis; Hepatitis C virus; Fibrogenesis; Parenchymal extinction; Portal thrombosis; Protein C

Core tip: Liver cirrhosis may be considered a pro-thrombotic condition despite it being associated with a low platelet count and deranged synthesis of clotting factors. When hepatitis C virus (HCV) is the etiological factor of liver cirrhosis, intrahepatic coagulation may

be enhanced by several direct actions of HCV on the clotting system, platelet aggregation, and altered anticoagulation. The excessive thrombin generation may be related to increased fibrogenesis both by a direct effect of thrombin on hepatic stellate cells and fibrosis related to ischemic parenchymal extinction.

INTRODUCTION

Liver damage in hepatitis C virus (HCV) infection includes a wide range of clinical entities. Chronic infection may lead to chronic hepatitis, with more or less marked steatosis and/or steatohepatitis, cirrhosis, and hepatocarcinoma. In advanced stages of HCV infection, recent research has pointed out the importance of thrombotic events and microcirculatory changes within the liver. As in other forms of cirrhosis, but probably with a higher prevalence, in HCV-infected patients thrombin formation with accompanying microthrombosis of portal vein radicles takes place, a phenomenon that may accelerate the natural history of the disease. The factors involved in this process, and the clinical relevance of this prothrombotic situation are reviewed in the present work.

HEMOSTATIC SYSTEM

Coagulant and anticoagulant pathways: A schematic overview

Hemostasis, in its wide sense, involves adhesion, aggregation, and activation of platelets (primary hemostasis), and the formation of a fibrin clot as the final product of an autocatalytic process initiated by the damaged endothelium. As shown in Figure 1, thrombin, a protein derived from the action of activated factor X on prothrombin, plays a central role. Thrombin promotes platelet activation and aggregation, and activates factor XI, factor VIII and factor V, creating a positive feed-back loop that underscores the need of a counter-regulatory system. This is composed by antithrombin, tissue factor pathway inhibitor, thrombomodulin, liver synthesized vitamin-K dependent protein C and its endothelial receptor, and protein S. Thrombomodulin is a transmembrane protein located on endothelial cells, in intimate connection with endothelial protein C receptor (Figure 2). Thrombomodulin acts as a thrombin receptor. Once thrombin and protein C bind to thrombomodulin

and protein C receptor, protein C becomes quickly activated by thrombin, a process highly dependent on thrombomodulin. Once activated, protein C binds to protein S. This complex inhibits activated factor V and factor VIII, leading to decreased thrombin generation (Figure 2). Thrombin generation is also quenched by the presence of antithrombin, a protease with many actions, also synthesized in the liver, and tissue factor pathway inhibitor (Figure 1), whose levels are not decreased, but even increased in cirrhosis[1]. Antithrombin strongly inhibits thrombin, but also inhibits the activated forms of factors XII, XI, X, IX and VII; plasmin and kallikrein, trypsin and C1[2-4]. Tissue factor pathway inhibitor forms a complex with factor Xa, inactivating it; and also blocking the tissue factor-factor VIIa complex. Like thrombomodulin, tissue factor inhibitor is an endothelial product[5] and, as discussed later, both may become altered in hepatitis C-dependent endothelial damage.

Platelets constitute another key component of hemostasis. Endothelial disruption exposes subendothelial collagen to the bloodstream, and this is followed by an avid binding of collagen to collagen-specific Ia/IIa platelet surface receptors. Another endothelial-derived multimeric protein, von Willebrand factor, firmly interacts with platelet glycoproteins Ib/IX/V and collagen fibrils. The function of this multimeric protein is controlled by a metalloprotease (ADAMTS 13), synthesized by several cells, including hepatic stellate cells and endothelial cells, among others[6]. Binding of collagen to glycoprotein VI receptor activate the platelets - a process also facilitated by thrombin which activates platelets by binding to other types of receptors, namely specific G protein receptors. Activated platelets release several products contained in their granules, including potent proinflammatory mediators, and finally aggregate and form a hemostatic plug. Aggregated platelets efficiently anchor fibrin, forming a plug that stops bleeding. Platelet membrane phospholipids bind to the gamma carboxy residues of activated factors IX and X, serving as a "platform" on which coagulation takes place. Calcium mediates this binding.

In order to avoid excessive thrombus formation, fibrinolysis is activated. This is also a tightly regulated process, by which an inactive protein - plasminogen - transforms into an active one - plasmin, which is able to destroy formed fibrin. A series of substances, such as tissue plasminogen activator, factor XIIa, and urokinase plasminogen activator transform plasminogen into plasmin, whereas several others block this effect. Indeed, plasminogen activator inhibitor, thrombin activatable fibrinolysis inhibitor (TAFI)[7], plasmin inhibitor, and histidine rich glycoprotein are all potent antifibrinolytic agents.

Thrombosis in HCV infection

There is considerable evidence that the hepatitis C
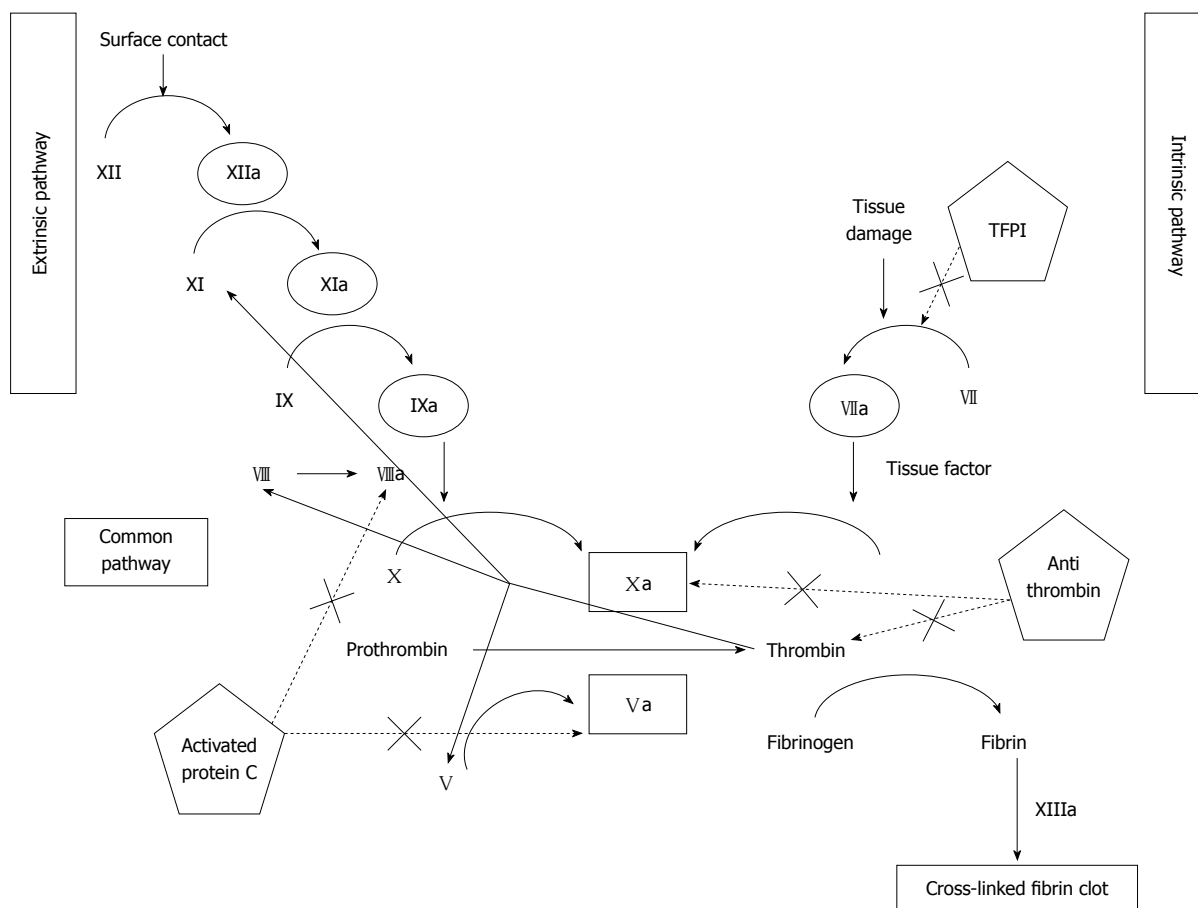
Figure 1 Coagulation cascade: Extrinsic, intrinsic, and common pathways. Coagulation factors are depicted in roman numerals with the suffix "a" denoting activated forms of the factors. Inhibitory pathways are shown in dotted lines. TFPI: Tissue factor pathway inhibitor.
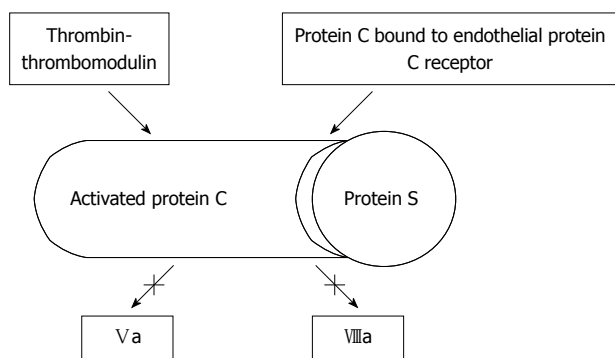
Figure 2 Anticoagulant effect of thrombomodulin, protein C, and protein S.

virus is able to activate hemostasis through several mechanisms, one of the main processes being HCV-induced endothelial damage and/or activation. Several clinical observations that are outlined below support this evidence (Figure 3).

In 1996, Prieto *et al*[8] reported an increased prevalence of anticardiolipin antibodies among HCV patients. This increased prevalence was related to prior thrombotic episodes, portal hypertension and thrombocytopenia. In a study on 201 patients, 124 of them with HCV infection, Biron *et al*[9] found a 33% prevalence of antiphospholipid antibodies that were

significantly associated with increased liver fibrosis assessed by METAVIR fibrosis score. A relationship between HCV infection and prothrombotic state was also evidenced by Enger *et al*[10] (2014), who found that HCV infection was associated with various kinds of thromboembolic diseases in a cohort of 22733 HCV infected individuals in the United States. The incidence rate ratio among non-cirrhotic HCV infected patients was 1.44 (95%CI: 1.31-1.58) for any thrombotic event, and reached 6.06 (95%CI: 2.04-18.01) for portal vein thrombosis. Wang *et al*[11] compared 3686 HCV-infected patients with 14744 subjects without HCV or HBV infection and followed them during more than 5 years. They found that HCV infection was associated with a hazard ratio of deep vein thrombosis of 1.96 (95%CI: 1.03-3.73). Chen *et al*[12] in 2013 found a higher prevalence of pulmonary hypertension among HCV patients, portal vein thrombosis (a feature commonly present in their series of HCV patients) being an independent factor. However, other authors have failed to find a relationship between antiphospholipid antibodies and thrombotic episodes in HCV patients[13,14], although antiphospholipid antibodies were more frequently observed among HCV patients. Already in 1995, Violi *et al*[15] performed a study on 18 patients with liver cirrhosis and 36 controls and found that
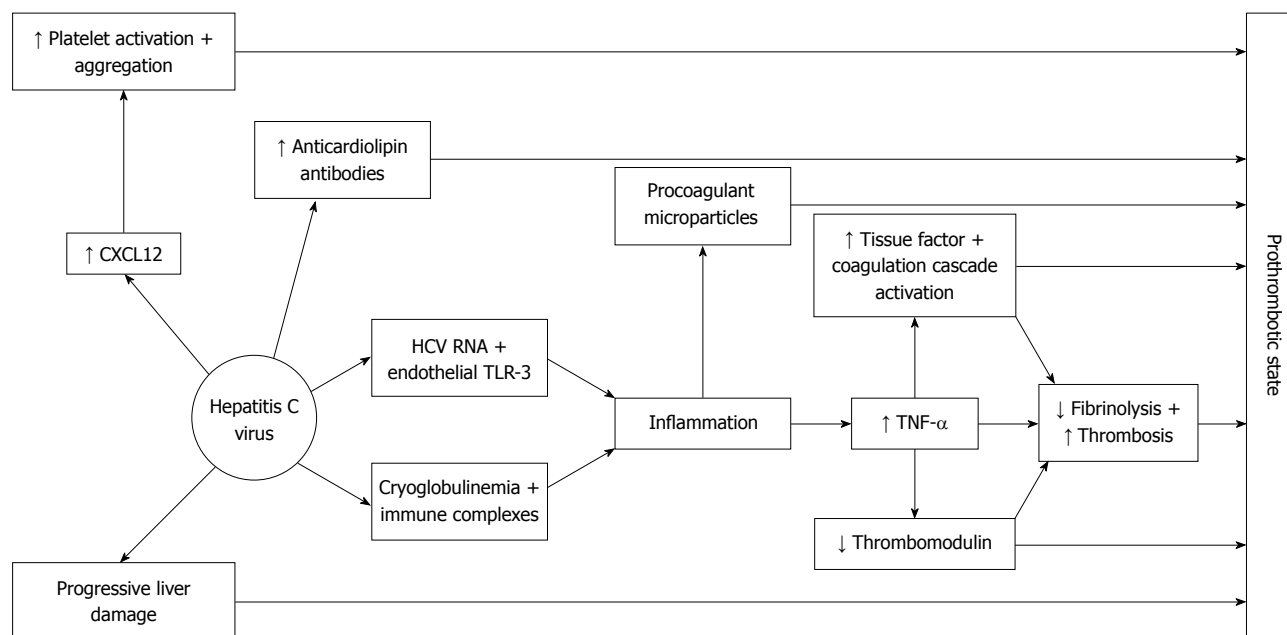
Figure 3 Hepatitis C virus has several effects on anticoagulant and procoagulant cascades, in addition to the effects on coagulation derived from liver cirrhosis development. (1) Hepatitis C virus (HCV) infection is associated with anticardiolipin antibodies which are related to thrombotic events; (2) Viral HCV RNA binds to toll-like receptors (TLR)-3 found in endothelial cells which leads to inflammation. Also, HCV infection is associated with cryoglobulinemia and thus immune complexes directed against viral RNA are formed. Inflammation generated by these two mechanisms lead to TNF-α secretion. TNF-α is an inducer of tissue factor expression, therefore exerting a prothrombotic effect by activating the coagulation cascade and also downregulates thrombomodulin expression; and (3) In HCV infection, CXCL12 is up-regulated in the endothelium of blood vessels formed in active inflammatory foci. CXCL12 is a potent promoter of platelet aggregation and adhesion.

venous thrombosis was associated with HCV infection, positive antiphospholipid antibodies, and increased rate of thrombin generation.

Main mechanisms involved

The mechanisms that may contribute to thrombosis in HCV infection include: systemic inflammation due to viral infection, direct infection of endothelial cells, viral-induced down regulation of physiological anticoagulant mechanisms, and alterations of fibrinolysis and thrombin generation dependent upon tissue factor generation by the infected endothelium[16]. The generation of antiphospholipid antibodies associated with HCV infection may also favor the generation of a procoagulant milieu[8], and immune complexes, usually in relation to cryoglobulinemia, may also trigger thrombotic phenomena[17].

Direct effect of HCV on anticoagulant and procoagulant pathways

Activation of tissue factor is of primary importance in the initiation of the coagulation cascade; and, as previously mentioned, damaged endothelium is the main source of tissue factor. Endothelial damage takes place in HCV infected patients, mainly by two main mechanisms. Firstly, today it is well known that HCV viral RNA binds to toll-like receptor (TLR)-3 in endothelial cells leading to inflammation[18] and generating enhanced expression of both tumor necrosis factor (TNF)-α and TNF receptor 2. In advanced stages of the

disease, endothelial cells also express the chemokine CXCL 12 that recruits immune cells[19]. Additionally, endothelial damage associated with cryoglobulinemia is due to a type 3 hypersensitivity reaction with formation of immune complexes of antibodies directed against viral RNA. These immune complexes activate endothelial cells[20]. Inflammation generated by either of these mechanisms is accompanied by TNF-α secretion. TNF-α is an inducer of tissue factor expression, therefore exerting a prothrombotic effect[21] and also down-regulates thrombomodulin expression[22]. Cytokines decrease fibrinolytic properties of the endothelial cells[23]. In addition, tissue factor is present in increased amounts in microparticles in patients with chronic pure HCV infection[24], contributing in this way to enhanced coagulation.

HCV infection and platelets

Hemostatic platelet function is also enhanced. In patients affected by HCV infection, CXCL12 is up-regulated in the endothelium of blood vessels formed in active inflammatory foci. It is elevated in the plasma of patients with marked fibrosis and avidly binds to CXCR4 overexpressed by liver infiltrating lymphocytes[19]. CXCL12 is a potent promoter of platelet aggregation and adhesion[25], and, indeed, increased platelet activation and aggregation have been described among HCV patients[26,27].

Perhaps this increased platelet aggregation may explain some striking features described in HCV

patients regarding platelet count. Thrombocytopenia is more marked in HCV-infected patients than among those affected by other forms of chronic liver disease in a similar stage of severity[28]. The reasons are not well understood[29,30], and there are reports in which thrombocytopenia improves after treatment with interferon alpha[31]. This effect is seen despite the fact that thrombocytopenia is a well known side effect of interferon alpha, which strongly suggests a direct causal relationship between thrombocytopenia and HCV infection. Theoretically, thrombocytopenia could be due to decreased production, but results regarding thrombopoietin levels are disparate. Español *et al*[32], in 2000, found normal values in 23 HCV patients with chronic hepatitis compared with 43 controls, but there are also reports pointing to decreased thrombopoietin levels in relation with progression of liver disease[33,34], and restoration of megakariopoiesis after successful liver transplantation[35]. These data suggest that liver function is essential for the maintenance of normal thrombopoietin levels, although it has been also reported that thrombopoietin may be degraded in excess by the enlarged spleen[36]. Thrombocytopenia could also be related to hypersplenism, but although splenic sequestration surely plays a role in advanced stages of liver disease, thrombocytopenia is already evident before spleen enlargement ensues. Antiplatelet antibodies have been described in HCV infection[37], but their pathogenetic role in thrombocytopenia is debatable[38]. However, in some studies the severity of the disease is accompanied by a progressive decrease of platelet production and an increase in platelet antibodies. Platelet count was inversely related to liver fibrosis and directly to viral load[39].

In any case, increased coagulation and decreased fibrinolysis, together with enhanced platelet aggregation may underlie the aforementioned well described relationship between HCV infection and venous thrombosis. When liver damage evolves during the natural history of chronic HCV hepatitis, further mechanisms add to those described.

Prothrombotic alterations associated with liver cirrhosis

Complications of liver cirrhosis probably account for most of the hospital admissions of patients with advanced HCV liver disease. As other forms of liver cirrhosis, cirrhosis in patients affected by chronic HCV infection is characterized by progressive fibrous tissue deposition in the liver that separates groups of hepatocytes forming nodules with distorted vascular architecture and variable degrees of necrosis and liver cell regeneration[40].

Two syndromes converge in this disease: liver failure and portal hypertension, leading to a constellation of clinical and laboratory alterations, some of them theoretically associated with increased bleeding risk. Among these, the most outstanding features related to portal hypertension include the development of oesophageal varices, hypertensive gastritis, hemorrhoids, and hypersplenism; secondary thrombocytopenia is usually found in these patients. Liver failure impedes correct synthesis of clotting factors, such as prothrombin or, in later stages, fibrinogen. Therefore, both syndromes may cause bleeding diathesis. However, in stark contrast, thromboembolic events are not unusual in cirrhosis[41,42], with an overall incidence of 0.8% of non portal vein thrombosis in a study on 2074 cirrhotic patients[43]. In that study, although 5 out of the 17 affected patients showed antiphospholipid antibodies, none of them showed mutation of factor V and/or prothrombin, so no other classic prothrombotic abnormality was identified. On the other hand, the incidence of portal vein thrombosis is by far higher among cirrhotics than among the general population, reaching prevalence values of 0.6%-5%, increasing up to 40% among patients with advanced disease, or 10%-25% according to other reports[44]. Local factors, such as venous stasis and portal hypertension-related endothelial dysfunction are clearly involved in this type of venous thrombosis, but it is worth of note that considering all the forms of venous thromboembolic disease together, cirrhotic patients showed an increased risk, that was more marked among patients with cirrhosis categorized as Child class C, despite a more deranged prothrombin activity[45].

Therefore, liver cirrhosis, despite a usually observed low platelet count and deranged synthesis of some clotting factors, may be considered a prothrombotic condition.

Anticoagulant and procoagulant pathways in cirrhosis

Independent of etiology, recent research has pointed out that in liver cirrhosis several alterations predispose to an increased thrombotic risk, especially at the portal vein.

Synthesis of antithrombotic proteins, such as antithrombin, protein C and protein S is more intensely deranged than that of procoagulant proteins. As mentioned earlier, protein C plays a major role in controlling coagulation. In its active form it degrades several coagulation factors, especially factor V, leading to a decrease in thrombin production. Decreased synthesis of protein C by an impaired liver function may favor ongoing thrombin formation[46]. Binding of thrombomodulin to protein C increases the speed of protein C activation. Interestingly, in a previous study we found raised levels of thrombomodulin among cirrhotics, in the face of decreased protein C, protein S and antithrombin[47]. However, thrombomodulin-bound thrombin also shows a prothrombotic effect, since it inhibits fibrinolysis by cleaving TAFI into its active form[48]. Therefore, raised thrombomodulin, *via* its action on TAFI, can be viewed as another factor potentially involved in the procoagulant milieu of liver cirrhosis.

Thrombin activation may be aggravated in some

situations in which anticoagulant pathways are further impaired. Factor Ⅴ Leiden is a common (2%-15% prevalence among Caucasians) autosomal dominant trait[49]. It carries a single mutation at position 506 that makes it resistant to the degradative action of activated protein C. As a consequence, the action of factor Ⅴa on thrombin synthesis increases, leading to a procoagulant state. Indeed, factor Ⅴ Leiden is associated with an increased risk of portal vein thrombosis both in patients with and without cirrhosis[50]-although there are studies that do not support this finding[51]. In addition, in patients with HCV infection who also bear factor Ⅴ Leiden polymorphism there is an increased rate of liver fibrous tissue deposition[52], whose underlying mechanisms will be discussed later. Poujol-Robert *et al*[53], in 2004, reported an increased odds ratio for cirrhosis among patients with HCV infection and factor V Leiden mutation, and Papatheodoridis *et al*[54] (2003) found that the presence of activated protein C resistance was associated with more intense fibrosis in patients with chronic viral hepatitis. Moreover, factor Ⅴ Leiden also carries an increased risk of fibrosis in other tissues, as shown by Xu *et al*[55] (2001) in pulmonary fibrosis that developed in bleomycin-treated mice carrying the factor Ⅴ Leiden mutation: both homozygous and heterozygous animals showed a nearly 40% increase in hydroxyproline excretion compared to wild-type mice.

Other factors may contribute to this pro-coagulant effect. Persistent or chronic inflammation is a thrombophilic condition, characterized by raised fibrinogen and factor Ⅷ, which are main contributors to this procoagulant milieu. Cirrhotics show raised levels of factor Ⅷ[56]. Also, cirrhotics have raised von Willebrand factor, which may favor a greater platelet adhesion[57]. Lipoprotein receptor-related protein is responsible for catabolism of factor Ⅷ. Its expression is decreased in cirrhotics[58]. In a similar fashion, ADAMTS-13, a metalloprotease involved in the catabolism of von Willebrand factor, is reduced in patients with liver cirrhosis[59]. Increased fibrinolysis related to decreased PAI-1 levels in relation to t-PA were also reported in cirrhotics[60], and a parallel deficiency in other mediators, such as TAFI, probably contributes[61]. It is currently accepted that hyperfibrinolysis may affect 30%-50% of cirrhotics with advanced disease[62].

Endothelial alterations of the portal vein radicles are well described in liver cirrhosis[63]. Endotoxaemia possibly plays a relevant role in endothelial alterations[64], independent on the eventual direct effects of HCV infection. As mentioned above, altered endothelium promotes coagulation by activation of tissue factor. In cirrhotics there is also an increase in the expression of several adhesion molecules, including platelet-endothelial cell adhesion molecule-1 (PECAM-1), L-selectin and P-selectin[65], and, as just mentioned, increased levels of von Willebrand factor[57].

Activated endothelial cells, as well as monocytes and platelets, also lead to the formation of microparticles that also carry tissue factor. In addition, platelet derived microparticles are able to transfer the GⅡb-Ⅲa platelet receptor to leukocytes, a feature which leads to the activation of the nuclear transcription factor kappa B, inducing gene transcription of proinflammatory mediators[66]. In addition platelet microparticles are able to carry factor Ⅴ[67]. Some studies point to an increased production of microparticles derived from leukocytes, lymphocytes, erythrocytes or even hepatocytes in liver cirrhosis[68]; despite some assertions[69], other researchers have failed to find raised platelet-derived microparticles in cirrhotic patients[70].

In summary, cirrhotics show more depressed levels of anticoagulants than those of procoagulants; although the role of microparticles in liver cirrhosis is unclear, portal hypertension-related endothelial damage and endotoxin-mediated cytokine activation, together with altered fibrinolysis in some cases, all contribute to the prothrombotic state of these patients, with increased thrombin formation.

Platelets are also altered in liver cirrhosis, both in number and function. Decreased platelet number may be due to splenic pooling due to portal hypertension, lower thrombopoietin levels, and increased consumption due to endotoxaemia, increased antiplatelet antibody production, and increased coagulation activation[69], but no significant bleeding takes place if platelet count is over 60000/fl[71]. Qualitative platelet alterations include defective adhesion[72], partially compensated by increased von Willebrand factor; decreased aggregation in response to normal stimuli, such as ADP, ristocetin, thrombin, collagen or epinephrine[73] and altered function[26] which progresses as liver function worsens. The aforementioned increased production of prothrombotic platelet-derived microparticles may compensate for these defects, so that hemostasis does not become significantly altered. On the contrary, portal thrombotic events may occur if a thrombopoietin receptor agonist (Eltrombopag) is administered[74], despite a median maximum platelet count of 148000/fl.

All these factors add to the previously mentioned direct effects of HCV infection, explaining why in HCV liver cirrhosis the thrombotic complications are more frequently observed than in cirrhosis of other etiologies.

CONSEQUENCES OF INCREASED THROMBIN FORMATION

Thrombin and fibrous tissue deposition: Direct effects on fibrogenesis

Thrombin not only plays a central role in the coagulation system. The importance of increased thrombin activation in patients with liver cirrhosis resides in the fact that thrombin may be directly involved in fibrogenesis (Figure 4). Thrombin exerts this action after binding to a group of receptors called protease activator receptors (PAR). There are four of such
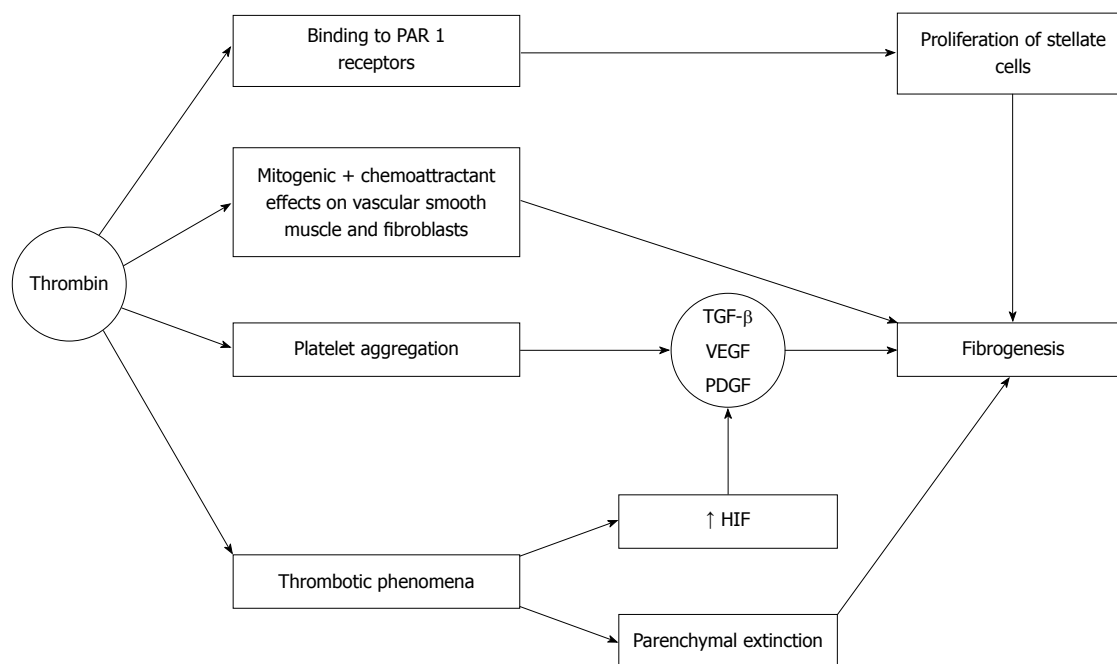
Figure 4 Effects on thrombin on fibrogenesis. Thrombin binds to PAR-1 receptors on hepatic stellate cells which leads to proliferaton and activation of these cells Thrombin also promotes platelet aggregation. Platelet alpha granules are rich in several growth factors, including TGF-β, which in turn promotes fibrogenesis. Vascular endothelial growth factor (VEGF) and PDGF also play contributory roles. Thrombin also exerts mitogenic and chemoattractant effects on vascular smooth muscle cells and fibroblasts. Finally, thrombotic phenomena also occur within the liver, leading to ischemic parenchymal injury, and substitution of parenchyma by fibrous tissue (the so called parenchymal extinction). When a clot provokes ischemia, VEGF, PDGF and TGF-β are activated probably *via* an increase in hypoxia-inducible-factor (HIF), which is raised in cirrhosis in relation to portal microthrombotic phenomena.

type of receptors (1-4), which become activated by several different proteases including thrombin (which activates PAR 1, 3, 4), and tryptase, which activates PAR-2, providing a rational basis to sustain the finding of liver fibrosis in the context of systemic mastocytosis[75]. PAR-1 receptors are present in the liver, and increase their expression in advanced liver disease[76] and along the transformation of stellate cells into myofibroblasts[77]. Indeed, hepatic stellate cells are one of the main cells in which these receptors become up-regulated in chronic liver disease. Binding of PAR with their ligands on stellate cells leads to proliferation and activation of these cells, that secrete monocyte chemoattractant protein 1 (MCP-1), increase deposition of extracellular matrix and the expression of receptors for platelet derived growth factor (PDGF) and transforming growth factor (TGF)-β[78]. In addition MCP-1 attracts monocytes, that produce tissue factor which contributes to more thrombin generation[79].

Another well known effect of thrombin is the ability to promote platelet aggregation. Platelet alpha granules are rich in several growth factors, including TGF-β, which in turn promotes fibrogenesis. Vascular endothelial growth factor (VEGF) and PDGF also play contributory roles. PDGF is a very strong mitogen for stellate cells and it also promotes fibrogenic activity by these cells[80]. PDGF upregulates the expression of matrix metalloproteinase (MMP)-2, MMP-9 and tissue inhibitor of metalloproteinase (TIMP)-1 and downregulates that of collagenase[40].

Thrombin also exerts mitogenic and chemoa-ttractant effects on vascular smooth muscle cells and fibroblasts[81]. Factor Ⅹa also activates PAR-1 and PAR-2, and may lead to fibrous tissue deposition. It was shown that incubation of fibroblasts with factor Ⅹa led to a 12.6-fold increase in TGF-β expression, an increase which was by far more intense than that elicited when the cells were stimulated by thrombin[82].

Therefore, thrombin, both directly and indirectly, together with other activated coagulation factors, may play a role in the progression of liver cirrhosis, *via* the described effect on fibrogenesis.

Thrombin and clot formation: Parenchymal extinction and fibrogenesis
In addition to thrombosis of major veins, microthrom-botic phenomena also occur within the liver, leading to ischemic parenchymal injury, and substitution of parenchyma by fibrous tissue (the so called parenchymal extinction[83]). This phenomenon consists in the ischemic collapse of hepatocytes between portal vein radicles and hepatic central venule, and becomes strongly exacerbated if congestive phenomena coexist, such as heart failure[84]. Coalescence of neighbouring tracts could lead to the formation of gross fibrous tracts and the evolution to cirrhosis[85]. When a clot provokes ischemia, VEGF, PDGF and TGF-β are activated[86] probably *via* an increase in hypoxia-inducible-factor (HIF), which is raised in cirrhosis due to increased portal resistance[87].

However, there are studies that do not support a pathogenetic role of portal vein thrombosis on progression of liver disease[88], but, as discussed above, factor V Leiden seems to accelerate progression of fibrosis in HCV infected patients. In other studies, other prothrombotic conditions such as hyperhomocysteinaemia[89] or mutations in factor XIII (both as an isolated finding or in combination with PAI-1 4G/5G mutation) also constitute a risk factor for an increased rate of liver fibrosis development in patients affected with HCV or chronic hepatitis B[90]. In other studies, the mutation associated with increased fibrosis progression rate was the prothrombin G20210 A mutation[91].

These data support the importance of micro-thrombotic phenomena in the progression of liver disease, especially in HCV-infected patients, in whom the endothelial changes promoted by HCV may be considered triggering factors, aggravated in later stages of the disease by the endothelial changes secondary to portal hypertension.

Therapeutic future prospects

Based on the aforementioned data, low molecular weight heparin has been advocated as a therapeutic option in patients with cirrhosis, with promising results: enoxaparin for 48 wk not only significantly prevented the development of portal vein thrombosis in patients with cirrhosis with Child-Pugh scores between 7 and 10, but it also associated with a decreased probability of decompensation of liver disease[92]. In that study, 18 out of 34 randomized to enoxaparin and 18 out of 36 in the placebo group were HCV-infected patients. Experimental research in rats has shown that treatment with heparin reduced severity of biochemical and histological changes induced in rats with oral carbon tetrachloride[93]. The possible role of anticoagulation in the treatment of advanced liver disease, especially in HCV-induced cirrhosis, is a promising idea that warrants confirmation[94].

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