Chapter 8

Summarizing discussion
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The main focus of the studies described in this thesis was to assess the clinical impact of gelatinase-type of matrix metalloproteinases MMP-2 and MMP-9 in several chronic liver diseases, with emphasis on orthotopic liver transplantation (OLT). In addition to this, we have investigated the interaction between MMPs and the fibrinolytic system during OLT. Finally, the influence of functional gene promoter polymorphisms of these MMPs was investigated.

A short general overview of the current knowledge of MMPs is provided in the introduction of this thesis (chapter 1). MMPs are the main degrading enzymes of extracellular matrix proteins and basement membranes, and they play an important role in the processes of tissue remodeling and repair in many physiological and pathological processes. A subgroup of the MMP family, the gelatinases MMP-2 and MMP-9, is thought to be involved in the degradation of matrix in the liver. MMP-2 can be expressed by many cell types in the liver, however, the hepatic stellate cell is suggested to be the main cellular source. The principal source of MMP-9 in the liver is the Kupffer cell, but MMP-9 can also be released from inflammatory cells, e.g., neutrophils. In the second part of chapter 1 the potential contribution to and role of MMP-2 and MMP-9 in liver fibrosis, hepatocellular carcinoma (HCC), ischemia/reperfusion (I/R) injury and acute rejection after OLT is described. Based on these findings the aims of this thesis are formulated.

In the first study of this thesis, as described in chapter 2, the clinical significance of serum MMP-2 and MMP-9 was assessed in 91 patients with several chronic liver diseases and in 60 controls. Serum levels of MMP-2 were significantly higher in patients with chronic liver disease compared to controls, and increased according to the progression of liver disease in patients with cirrhosis. There was a strong correlation between MMP-2 and serum markers of liver dysfunction (bilirubin, albumin, and prothrombin time). Inversely, serum MMP-9 levels had an opposite correlation with these parameters, and were found to be decreased in patients with chronic liver disease as compared to controls. However, the MMP-2 and MMP-9 serum levels in an individual patient were not found to be useful markers for liver function because of a wide overlap in levels between the different Child-Pugh stages.

In patients with HCC, MMP-2 levels were significantly higher than in controls, but comparable to patients with chronic liver disease. MMP-9 yielded no significant differences between patients with or without HCC and controls. Due to a considerable overlap of serum MMP-2 and MMP-9 levels in patients with chronic liver disease with or without HCC, these parameters can not be used as diagnostic markers for HCC in the context of chronic liver disease.

Next we assessed the evolution of plasma MMP-2 and MMP-9, and their inhibitors TIMP-1 and TIMP-2, in 24 patients during OLT (chapter 3). Plasma TIMP-1, TIMP-2 and MMP-2 levels gradually decreased during transplantation. Approximately two-third of total MMP-2 appeared to be in its activatable proMMP form. No release of MMP-2 from the graft could be detected. In contrast, plasma levels of MMP-9 increased sharply during OLT. Peak MMP-9 levels of about eight times above baseline were found at 30 minutes after reperfusion. Most MMP-9 appeared to be in its active/inhibitor-complexed form. There was a significant correlation between plasma MMP-9 and tissue-type plasminogen activator (t-PA) levels, but not with tumor necrosis factor-α. In conclusion, OLT is associated with a sharp increase of MMP-9 during the anhepatic and postreperfusion periods, which coincided with the changes in t-PA, suggesting common underlying mechanisms of activation.
It is known that OLT is associated with increased fibrinolytic activity due to elevated plasmin generation, which might activate some MMPs. Therefore the effect of serine-protease inhibition by aprotinin was investigated. No significant differences in MMP-2 and MMP-9 were observed between patients treated with aprotinin and placebo. Also, the composition of these MMPs was not altered by the use of aprotinin, suggesting that serine–protease/plasmin-independent pathways are responsible for MMP activation during OLT.

MMPs have been suggested to play an important role in I/R injury during OLT. In patients with more severe I/R injury the MMP-9 concentration, particularly of the active/inhibitor-complexed form, remained high postreperfusion compared to patients with no or mild I/R injury. The decrease in MMP-2, TIMP-1 and TIMP-2 during OLT occurred irrespective of I/R injury. Therefore, only MMP-9 seems to be involved in early I/R injury during human liver transplantation and controlling local MMP-9 may thus be a target for reducing this injury during liver transplantation.

A subgroup of the patients described in chapter 2 underwent an OLT in the Leiden University Medical Center because of severe liver dysfunction and/or HCC. In this group of 33 patients the changes in serum MMP-2 and MMP-9, and their composition, were assessed with respect to the late phase of I/R injury after OLT (chapter 4). Both MMP-2 and MMP-9 serum levels, and isoform composition, were found to be comparable two days after OLT between patients with more severe I/R injury and those with absent to mild I/R injury. Therefore, serum MMP-2 and MMP-9 do not seem to play a major role in the late phase of hepatic I/R injury after OLT.

The time course of serum MMP-2 and MMP-9 during one year after OLT, with emphasis on acute allograft rejection, is described in chapter 5. The extracellular matrix may be an important target in the process of acute rejection after OLT and we demonstrated significantly higher total and active/inhibitor-complexed MMP-9 in patients with rejection compared to those without rejection. Immunohistochemical staining of liver biopsies at one week after OLT showed increased numbers of MMP-9 positive inflammatory cells, notably neutrophils and lymphocytes, in the portal triads of patients with rejection.

Serum MMP-2 levels in patients before OLT were significantly higher compared to controls. Also, the MMP-2 content of cirrhotic liver specimens was significantly higher compared to normal liver, indicating an important role of MMP-2 in the development of fibrosis. After OLT serum MMP-2 decreased about 50% but did not return to levels comparable with controls. This latter suggest the presence of persistent extracellular matrix remodeling in some of these patients and reflects probably a multifactorial cause, e.g. return of the original disease in the liver (hepatitis C), low-grade chronic allograft rejection or inflammation.

The significance of blood collection as a preanalytical determinant for MMP measurement was studied by determining MMP-2 and MMP-9 in simultaneously collected serum, sodium-heparin and EDTA plasma samples in 7 patients at 6 different time points after OLT, as described in chapter 6. EDTA plasma samples were found to have a lower MMP level compared with the serum and heparin-plasma levels. The correlation in the MMP-levels between the different analytes were highly significant and the pattern of the MMP-levels over time were very similar for the different analytes. It was concluded that the type of preanalyte used might be relevant to the obtained absolute MMP level but less important in the study of the dynamics of changes in the MMP parameter after OLT.
Chapter 7 reports the study on the influence of the −1306 C/T MMP-2 and −1562 C/T MMP-9 gene promoter polymorphisms in chronic liver disease and transplantation. The MMP-2 and MMP-9 protein expression in serum appeared to be independent of the MMP genotype in our total study population. However, in the patients with cirrhosis there was a clear relationship between the higher MMP-2 serum levels with advanced Child-Pugh stage and a more frequent wild-type −1306 CC genotype of MMP-2. In association, a more frequent −1562 CT MMP-9 genotype with an increased serum level was found in the chronic liver disease patients without cirrhosis. The development of late phase I/R-injury or rejection after OLT, however, was found to occur unrelated to the MMP-2 and MMP-9 genotype of the donor, the recipient or their MMP-genetic mismatch.

In conclusion, the studies in this thesis describe the clinical impact of MMP-2 and MMP-9 in chronic liver disease and orthotopic liver transplantation. Stellate cells are most likely the key source of MMP-2 and are known to be actively involved in the alteration of extracellular matrix. The MMP-2 content of cirrhotic liver was higher compared to controls, and serum MMP-2 correlates positively with markers of liver dysfunction. These findings indicate, in concert with other recent publications (2;3), an important role of MMP-2 in the development and persistence of liver fibrosis. However, in an individual patient, the serum MMP-2 level cannot be used as a single marker for liver function or fibrosis/cirrhosis. Recent studies suggest that the combination of several serum markers related to factors involved in extracellular matrix remodeling and fibrosis, including MMP-2, MMP-9 and TIMP-1, may distinguish between patients without fibrosis and with advanced liver disease.(4-6)

End-stage chronic liver disease is often complicated by HCC. In contrast to the earlier study by Hayasaka, et al.,(7) suggesting that serum MMP-9 could be used as a diagnostic marker for HCC, our study clearly demonstrated that serum MMP-9 levels were the same in chronic liver disease patients with or without HCC and thus not discriminative for this end-stage complication. The search for serum markers of HCC, e.g. by serum proteomic profiling through mass spectrometry, has become an important research issue. This is illustrated by a very recent study which revealed that an MMP-cleaved fragment of vitronectin, a glycoprotein involved in cell adhesion and matrix remodeling, identifies HCC in patients with chronic liver disease.(8)

Elevated serum levels of MMP-9, probably derived from neutrophils and Kupffer cells, were found in patients with acute allograft rejection after OLT. MMPs have also been implicated in cold preservation injury to sinusoidal endothelial cells and we have demonstrated high plasma MMP-9 levels in the post-reperfusion period, especially in patients with more severe I/R injury. Therefore, MMP-9 seems to be a key mediator of early I/R-injury after OLT. The impact of MMP-9 was recently confirmed in a study on cold preservation injury using genetically deleted MMP-9/KO mice, which were found to have considerably less damage than the wild-type mice.(9) Although liver preservation solutions already contain cryptic MMP inhibitors (10), it remains speculative whether further pharmacological MMP inhibition may prohibit these complications of liver transplantation, due to unfavorable side effects upon chronic application and given the fact that MMPs are not only involved in the injury but also in the regeneration of the liver.(11;12)

Finally, the observation that the MMP-2 and MMP-9 genotypes are associated with their serum levels in relation to liver cirrhosis merits further studies in larger groups of patients with different stages of chronic liver disease and perhaps also other MMPs.
References