Plasma MMP–2 and MMP–9 and their inhibitors TIMP-1 and TIMP-2 during human orthotopic liver transplantation

The effect of aprotinin and the relation to ischemia/reperfusion injury

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Summary

Uncontrolled activation of matrix metalloproteinases (MMPs) can result in tissue injury and inflammation, yet little is known about the activation of MMPs during orthotopic liver transplantation (OLT). OLT is associated with increased fibrinolytic activity due to elevated plasmin generation. The serine-protease plasmin not only causes degradation of fibrin clots but is also thought, amongst others, to play a role in the activation of some matrix metalloproteinases. We therefore studied the evolution of MMP-2 and -9 plasma concentrations during OLT and the effect of serine-protease inhibition by aprotinin on the level and activation of these MMPs. In a group of 24 patients who participated in a randomized, double-blind, placebo-controlled study we determined serial MMP-2 and -9 plasma levels during transplantation using ELISA (total MMP), activity assays (activatable MMP) and zymography. In addition, the MMP-inhibitors TIMP-1 and TIMP-2 were assessed by ELISA. The putative regulating factors tumor necrosis factor alpha (TNF-α) and tissue-type plasminogen activator (t-PA) were assessed as well. Patients were administered high-dose aprotinin, regular-dose aprotinin or placebo during surgery. Plasma TIMP-1, TIMP-2 and MMP-2 level gradually decreased during transplantation. Approximately two-thirds 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 the anhepatic and postreperfusion periods. 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. No significant differences were observed between the three treatment groups. However, in patients with more severe ischemia/reperfusion (I/R) injury the MMP-9 concentration, particularly of the active/inhibitor-complexed form, remained high at 120 minutes postreperfusion compared to patients with no or mild I/R injury. The decrease in plasma levels of MMP-2, TIMP-1 and TIMP-2 during OLT occurred irrespective of the severity of the I/R injury. There was a significant correlation between MMP-9 and t-PA levels, but not with TNF-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. MMP-2, TIMP-1 and TIMP-2 gradually decreased during OLT. The composition of these MMPs was not altered by the use of aprotinin, suggesting that serine-protease/plasmin-independent pathways are responsible for MMP regulation during OLT. In addition, only MMP-9 seems to be involved in I/R injury during human liver transplantation.

Keywords

Aprotinin, ischemia/reperfusion injury, liver transplantation, matrix metalloproteinases, plasmin

Introduction

Matrix metalloproteinases (MMPs) are a group of more than 20 zinc-binding proteolytic enzymes that are collectively responsible for the degradation of extracellular matrix proteins and basement membranes (1). They play a role in several processes like angiogenesis, tumor invasion, and tissue regeneration and remodelling. Uncontrolled expression of MMPs can result in tissue destruction and inflammation. MMPs can be subdivided into four main classes: collagenases, gelatinases, stromelysins, and membrane-type (MT)-MMPs (2). MMP-2 (gelatinase A) and MMP-9 (gelatinase B) are considered to be of particular relevance to the liver, as they can degrade type IV collagens and fibronectin, two main components of the space of Disse. We recently showed that patients with a chronic liver disease have very high serum levels of MMP-2, particularly those in need of a liver transplantation, as opposed to marginally decreased levels of MMP-9 (3). MMP-2 and MMP-9 are presumed to play a role in the initiation of cold storage injury of the sinusoidal endothelial cells during ischemic preservation and in the subsequent reperfusion injury of liver grafts, which might contribute to initial poor function after orthotopic liver transplantation (OLT) (4-6). OLT is associated with an inflammatory response and increased fibrinolytic activity, leading to a ‘pro-lytic state’, especially during the anhepatic and postperfusion period (7-9). Several proteolytic cascades have been shown to play a role in the development of this ‘lytic state’, of which the generation of plasmin via the tissue-type plasminogen activator (t-PA) pathway is considered to be clinically most relevant (8). During the anhepatic and postperfusion period in particular, t-PA plasma levels are significantly increased, leading to the conversion of plasminogen into plasmin and subsequent degradation of fibrin clots and increased bleeding (8-10). Excessive plasmin formation does not only lead to premature degradation of fibrin clots, but may also have an important role in the activation of some MMPs (2, 11). In general, MMPs are secreted as latent pro-enzymes, which must first be cleaved to active forms before degradation of matrix proteins can be initiated (12). There are several pathways to activate proMMPs. Activation of proMMP-2 involves cleavage by MT1-MMP, yielding an intermediate form that may be activated by plasmin (13). Activation of proMMP-9 may occur directly via plasmin or via plasmin-independent mechanisms (14). Plasmin therefore may play an important role in the in vivo activation of proMMPs during OLT. Free, active MMP is normally not detectable in the circulation, as it is rapidly inactivated by specific naturally occurring tissue inhibitors of metalloproteases (TIMPs) and α2-macroglobulin.

Aprotinin, a strong inhibitor of plasmin and other serineproteases (15), has been shown to significantly reduce blood loss during OLT (16). Extensive research has contributed to a better understanding of the mechanism underlying the activation of the fibrinolytic system during OLT (17). However, little is known about the activation of MMPs during OLT despite the clear interactions with the fibrinolytic cascade (2). The aim of this present study was to evaluate the changes in plasma MMP-2, MMP-9, TIMP-1 and TIMP-2 concentrations during human OLT and to assess the in vivo effect of aprotinin on these MMP parameters.

Patients and methods

Patients, study protocol and transplant variables

Twenty-four consecutive patients (16 male; median age 47 years [range: 18-68]), who underwent OLT in one of the participating centers of the larger European Multicenter Study on the use of Aprotinin in Liver Transplantation (EMSALT) (17), were included in this study. Exclusion criteria for this study were pediatric patients (age < 18 years), patients with a history of thromboembolic disorders or malignancies, patients with previous exposure to aprotinin and patients with previous OLT. The ethical committee approved the study protocol and written informed consent was obtained for each patient. Patients were randomized into three different groups: high-dose aprotinin (n=8), regular-dose aprotinin (n=8), or placebo (n=8). Patients in the high-dose group were administered a loading dose of 2 × 10^6 KIU aprotinin (Trasylol®, Bayer AG, Leverkusen, Germany) during induction of anesthesia, followed by a continuous infusion of 1 × 10^6 KIU per hour and an additional 1 × 10^6 KIU 30 minutes before reperfusion. The regular-dose group was administered a loading dose of 2 × 10^6 KIU aprotinin, followed by 0.5 × 10^6 KIU aprotinin per hour. The placebo group was administered identical volumes of a 0.9% sodium chloride solution, provided in identical bottles. In all 3 groups, infusion of study medication was discontinued 2 hours after graft reperfusion. All donor livers were perfused and stored in the University of Wisconsin (UW) preservation solution. The three treatment groups were comparable for age, sex, etiology and severity of liver disease. No significant differences were observed in cold ischemia time.

The degree of hepatocellular injury was evaluated by postoperative measurement of aspartate aminotransferase (AST). The AST was determined at 7 consecutive days after OLT and measured by routine methods. In this study patients were classified into 2 groups depending on whether the serum AST peak during the first week after OLT was lower than 1,500 IU/L (no or mild I/R injury) or higher than 1,500 IU/L (more severe I/R injury), respectively (18, 19).

Evaluation of MMP-2, MMP-9, TIMP-1 and TIMP-2 plasma concentrations

Blood samples were collected from an unheparinized arterial line at 7 standardized time points during transplantation: before
transplantation (I), after induction of anesthesia (II), 10 minutes before the end of the pre-anhepatic period (III) and anhepatic period (IV), and 5 (V), 30 (VI) and 120 (VII) minutes postreperfusion. Blood was collected into polystyrene test tubes, containing trisodium citrate (0.129 M, 1/10 vol./vol.), immediately placed on melting ice and centrifuged (2,000 g, 4°C, 30 minutes) within 30 minutes. Plasma samples were snap frozen and stored in small aliquots at -70°C until further processing. Before use, plasma was thawed rapidly in a water-bath at 37°C and put on ice.

Total plasma MMP-2 and MMP-9 protein concentrations were determined at all 7 time points using our highly specific enzyme-linked immunosorbent assays (ELISAs), which measure the grand total of pro-enzyme, active- and inhibitor-complexed forms of the respective MMP, as described previously (20-22).

MMP-2 and MMP-9 enzymatic activities were determined using specific biochemical immunosorbent activity assays (BIA) (21-23). Free, active MMPs, however, cannot be detected in blood samples because of rapid complex formation with specific inhibitors in the circulation. Activatable proMMP-2 and proMMP-9 was measured at 3 or 4 time points (I, IV, VI and VII). Briefly, 96-well plates were coated as in the MMP-ELISA. Samples at various dilutions in assay buffer were added and incubated overnight at 4°C. ProMMP was activated by incubation with 0.5 mM p-aminophenylmercuricacetate and subsequently detection reagent was added, consisting of modified pro-urokinase and peptide substrate S-2444, after which the absorption over time was measured at 405 nm. The activatable proMMP-2 and proMMP-9 concentrations were calculated from the same standards as used in the ELISA. Finally we calculated active/inhibitor-complexed MMP-2 and MMP-9 levels from each individual sample by subtracting the level of activatable proMMP obtained by BIA from the grand total MMP level obtained by ELISA, to have an approximation of the active/inhibitor-complexed forms. The latter including TIMP-proMMP complexes, unique features of proMMP-2 and proMMP-9 (1).

In addition, gelatin zymography was performed on plasma samples at 3 time points (I, IV and VI) with quantification of specific activities by densitometry of the lysis bands, as described previously (21, 24).

TIMP-1 and TIMP-2 concentrations were determined by a quantitative two site ELISA sandwich format, using a commercial assay kit (Quantikine™, R&D Systems, Abingdon, UK). The concentration of inhibitor in a sample was determined by interpolation from a standard curve.

**Evaluation of t-PA and TNF-α**

The t-PA antigen was measured using a commercially available kit (TintElize t-PA; Biopool AB, Umeå, Sweden). Since the presence of aprotinin in the plasma samples interferes with the measurement of t-PA activity when conventional methods are used, we adopted modified bio-functional immunosorbent assays for the measurement of t-PA activity (Chromolize t-PA, Biopool AB). In this assay, microtitre wells are coated with monoclonal t-PA antibodies, which capture t-PA present in the plasma without blocking its active site. Contaminating proteins (e.g., aprotinin) are removed by washing, before the addition of a mixture of plasminogen, t-PA activity promoters and a plasmin-sensitive chromogenic substrate. With this t-PA activity assay only free and active t-PA is measured, whereas the total amount (free t-PA and t-PA bound to its inhibitor PAI-1) is measured by the t-PA antigen assay (25).

TNF-α plasma levels were measured using the Quantiglo® Human TNF-α Chemiluminescent Immunoassay (R&D Systems, Minneapolis, MN USA), a quantitative sandwich enzyme immunoassay, according to the manufacturer’s instructions (standard detection range 0.7 pg/ml to 7,000 pg/ml).

**Statistical analysis**

All variables are expressed as mean ± SEM. Statistical analysis was performed using SPSS 10.0 for Windows (SPSS Inc., Chicago, IL, USA). Comparisons among the three groups were made by means of the Kruskall-Wallis test, Mann-Whitney U test or Chi-square test for non-parametric data. The Wilcoxon signed ranks test for paired data was used to analyze changes within one group. The correlation (r) was evaluated by the Spearman rank correlation test for nonparametric data. P-values < 0.05 were considered to be statistically significant.

**Results**

### MMP-2 concentration during liver transplantation

The intraoperative course of MMP-2 plasma concentration during OLT is shown in Figure 1 (A-C). At induction of anesthesia mean plasma concentration of total MMP-2 protein was approximately 750 ng/ml. During transplantation, levels decreased gradually to approximately 400 ng/ml at 2 hours after graft reperfusion in all three groups (Fig. 1A). There was no increase of total MMP-2 protein after graft reperfusion, which suggests no release of MMP-2 from the donor liver. A similar decreasing pattern was found for the activatable pro-enzyme form of MMP-2 (Fig. 1B). Analysis of the ratio between pro-enzyme and active/inhibitor-complexed forms showed that approximately two-thirds of all MMP-2 was present in its activatable pro-enzyme form. The estimated amount of active/inhibitor-complexed MMP-2 in both aprotinin-treated groups decreased approximately two-thirds of all MMP-2 was present in its activatable pro-enzyme form. The estimated amount of active/inhibitor-complexed MMP-2 in both aprotinin-treated groups decreased approximately two-thirds of all MMP-2 was present in its activatable pro-enzyme form. The estimated amount of active/inhibitor-complexed MMP-2 in both aprotinin-treated groups decreasing to approximately 400 ng/ml at 2 hours after graft reperfusion in all three groups (Fig. 1A). There was no increase of total MMP-2 protein after graft reperfusion, which suggests no release of MMP-2 from the donor liver. A similar decreasing pattern was found for the activatable pro-enzyme form of MMP-2 (Fig. 1B). Analysis of the ratio between pro-enzyme and active/inhibitor-complexed forms showed that approximately two-thirds of all MMP-2 was present in its activatable pro-enzyme form. The estimated amount of active/inhibitor-complexed MMP-2 in both aprotinin-treated groups decreased significantly during OLT (Fig. 1C). Comparing the three treatment groups with each other, however, yielded no significant differences.

Gelatin zymography was performed at 3 time points (Table 1). In all 3 groups of patients proMMP-2, as assessed by
zymography, showed a similar pattern as observed with the BIA, i.e., a decrease during OLT. There were no significant differences between the 3 treatment groups at all time points. Overall, the correlation between ELISA, BIA and zymography results for MMP-2 was $0.44 < r < 0.53$ ($p<0.0005$). Free/active MMP-2 was not detectable by BIA or zymography.

**MMP-9 concentration during liver transplantation**

The intraoperative course of MMP-9 plasma concentrations is shown in Figure 1 (D-F). Mean total MMP-9 protein concentrations were approximately 50 ng/ml preoperatively and at induction of anesthesia. During the anhepatic and early postreperfu-
sion period total MMP-9 plasma concentrations increased approximately eight-fold, reaching a peak of approximately 400 ng/ml at 30 minutes after graft reperfusion (Fig. 1D). Plasma concentrations of activatable proMMP-9 were relatively low. Although these proMMP-9 levels increased during the anhepatic and postreperfusion stages, this occurred to a much lower extent than the increase in total MMP-9 protein (Fig. 1E). This suggests that the sharp increase of MMP-9 was mainly due to an increase in active/inhibitor-complexed MMP-9 (Fig. 1F).

No significant differences were observed comparing the three treatment groups among each other.

ProMMP-9 as determined by zymography demonstrated a similar pattern to the proMMP-9 in the BIA, with an increase during OLT (Table 1). Overall, there was a good correlation between ELISA, BIA and zymography results for MMP-9 (0.41 < r < 0.85; p<0.0005). Furthermore, evaluation of the zymographic results of MMP-9 revealed that it was mainly in the pro-enzyme form with no significant differences between the 3 treatment groups. Free/active MMP-9 was very low at zymography and absent in the BIA.

Plasma t-PA antigen concentrations and t-PA activity have been previously reported in detail (25). Both showed a similar pattern during OLT with an increase from baseline levels of 20.8 ng/ml and 1.1 mIU/ml, respectively, during the anhepatic period with peak values of 41.0 ng/ml and 3.7 mIU/ml after reperfusion and a subsequent decrease to normal values at 2 hours after reperfusion. Although there were no significant differences in t-PA activity between the three treatment groups, t-PA antigen was significantly higher in the placebo group compared to the high-dose aprotinin group during the early postreperfusion stage (25). We found a positive correlation between MMP-9 ELISA with t-PA antigen (r: 0.26; p=0.001, n=160) and active/inhibitor-complexed MMP-9 with t-PA activity (r: 0.38; p<0.01, n=68).

The mean TNF plasma concentrations during transplantation ranged between 4.1 and 8.7 ng/ml and there were no differences between the three groups. There was no significant correlation between MMP-9 and TNF-α protein concentrations (r: 0.14; p=0.07, n=159).

**TIMP-1 and TIMP-2**

In all three groups of patients TIMP-1 decreased significantly (p<0.05) during the anhepatic period, and decreased somewhat further after reperfusion (Table 2). In contrast, TIMP-2

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### Table 1: Plasma proMMP-2 and proMMP-9* during OLT by zymography.

<table>
<thead>
<tr>
<th>Time point†</th>
<th>High-Dose Aprotinin (n=8)</th>
<th>Regular-Dose Aprotinin (n=8)</th>
<th>Placebo (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ProMMP-2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>185 ± 38</td>
<td>141 ± 24</td>
<td>169 ± 41</td>
</tr>
<tr>
<td>IV</td>
<td>137 ± 30</td>
<td>87 ± 15‡</td>
<td>121 ± 26</td>
</tr>
<tr>
<td>VI</td>
<td>97 ± 18‡</td>
<td>78 ± 25‡</td>
<td>80 ± 16‡</td>
</tr>
<tr>
<td>ProMMP-9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>36 ± 11</td>
<td>27± 6</td>
<td>34 ± 12</td>
</tr>
<tr>
<td>IV</td>
<td>63 ± 30</td>
<td>43 ± 7</td>
<td>75 ± 30</td>
</tr>
<tr>
<td>VI</td>
<td>72 ± 18</td>
<td>108 ± 30‡</td>
<td>65 ± 10</td>
</tr>
</tbody>
</table>

*Data are mean ± SEM in arbitrary units; †, time points: before transplantation (I), anhepatic period (IV), and 30 minutes post-reperfusion (VI); ‡, p < 0.05 compared with baseline (time point I).

### Table 2: Plasma TIMP-1 and TIMP-2* during OLT.

<table>
<thead>
<tr>
<th>Time point†</th>
<th>High-Dose Aprotinin (n=8)</th>
<th>Regular-Dose Aprotinin (n=8)</th>
<th>Placebo (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TIMP-1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>255 ± 57</td>
<td>220 ± 48</td>
<td>357 ± 111</td>
</tr>
<tr>
<td>IV</td>
<td>149 ± 24‡</td>
<td>121 ± 17‡</td>
<td>158 ± 23‡</td>
</tr>
<tr>
<td>VI</td>
<td>111 ± 7‡</td>
<td>106 ± 21‡</td>
<td>134 ± 16‡</td>
</tr>
<tr>
<td>TIMP-2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>109 ± 9</td>
<td>96 ± 13</td>
<td>107 ± 14</td>
</tr>
<tr>
<td>IV</td>
<td>104 ± 16</td>
<td>86 ± 7</td>
<td>93 ± 15</td>
</tr>
<tr>
<td>VI</td>
<td>75 ± 7‡</td>
<td>63 ± 5‡</td>
<td>68 ± 8‡</td>
</tr>
</tbody>
</table>

*Data are mean ± SEM in ng/ml; †, time points: before transplantation (I), anhepatic period (IV), and 30 minutes post-reperfusion (VI); ‡, p < 0.05 compared with baseline (time point I).
showed a significant (p<0.05) decline only after reperfusion. There were no significant differences between the three treatment groups at the different time points. We found statistically significant, negative correlations between total MMP-9 ELISA and TIMP-1 (r: -0.29; p<0.05) and TIMP-2 (r: -0.41; p<0.0005). In contrast, total MMP-2 ELISA showed a strong positive correlation with both TIMP-1 (r: 0.37; p<0.0005) and TIMP-2 (r: 0.50; p<0.0005).

**MMPs in relation to I/R injury**

Five patients, three of whom received the high dose and one the regular-dose of aprotinin, attained a maximum AST value of 1,500 IU/L or higher during the first week after OLT, indicative for more severe I/R injury. Peak AST plasma values remained below 1,500 IU/L in the remaining 19 patients. The cold ischemia time did not differ between both groups. All patients showed improvement of liver function, i.e., decreasing AST, after the third post-operative day and no retransplantation was needed due to primary non-function.

During the anhepatic phase and early postreperfusion period total MMP-9 protein levels increased in both I/R groups. In the group of patients with more severe I/R injury the mean MMP-9 peak at 30 minutes postreperfusion (462 ± 124 ng/ml) was higher and showed no decrease at 120 minutes postreperfusion (451 ± 98 ng/ml), in contrast to the group of patients with no or mild I/R injury where MMP-9 did decrease significantly between 30 and 120 minutes postreperfusion (386 ± 77 and 272 ± 75 ng/ml, respectively). The MMP-9 concentration at 120 minutes postreperfusion differed significantly (p < 0.05) between both groups. The activatable proMMP-9, as determined by BIA, showed a comparable pattern in both groups. Therefore, the active/inhibitor-complexed MMP-9 concentration at 120 minutes postreperfusion was higher in patients with more severe I/R injury (424 ± 97) compared to the group of patients with no or mild I/R injury (253 ± 73; p < 0.05). Moreover, the patients in the latter group showed a significant decrease in the active/inhibitor-complexed MMP-9 concentration between 30 and 120 minutes postreperfusion, while in patients with more severe I/R injury it remained high.

Total protein, activatable pro-enzyme, as well as active/inhibitor-complexed MMP-2 plasma levels, gradually decreased during OLT, without major differences between the groups of patients in relation to I/R injury. Similarly, TIMP-1 and TIMP-2 were not significantly different at all three time points in the group of patients with or without I/R injury (data not shown).

**Discussion**

There are several novel findings in the present study. Firstly, we have shown that OLT is associated with a significant increase of MMP-9, especially during the anhepatic and postreperfusion stages, similar to that of t-PA, and not related to TNF-α. In contrast, MMP-2, TIMP-1 and TIMP-2 concentrations decrease during the operation. Secondly, we have shown that the enhanced MMP-9 level sustains in patients with more severe I/R injury. Thirdly, the composition of MMP-2 and MMP-9 during OLT is not affected by aprotinin, suggesting that serine-protease-independent mechanisms are responsible for their activation.

OLT is associated with a well-documented activation of the plasminogen/plasmin system (7-9). The changes in MMP-9 plasma concentration we observed during transplantation are very similar to those of other proteolytic enzymes, like t-PA and complement C3d-antigen. Moreover, we found a significant correlation between MMP-9 and t-PA, suggesting common underlying mechanisms of activation. The changes in t-PA have been explained by the absence of clearance by the liver during the anhepatic period, followed by a release from the graft in the early postreperfusion period (7, 9). Restoration of clearance by the transplanted liver is believed to explain the rapid decrease thereafter. Upadhya et al. (4) have shown that the first effluent of human donor livers after cold storage also contains high levels of MMPs, especially MMP-9. Therefore, it is likely that the 8-fold increase in MMP-9 observed during the OLT procedure in our study is at least partly explained by release from the donor liver. Another explanation for the increase in MMP-9 during the anhepatic period could be the combination of surgical stress and ischemia of the splanchnic circulation after clamping of the portal vein during the anhepatic period. Others have found elevated plasma levels of MMP-9 in patients suffering from acute ischemic stroke (26), and in patients with acute coronary syndromes (27). In addition, it has been previously shown that the cytokine-inducible MMP-9 is secreted by many cell types involved in the inflammatory response, including macrophages, neutrophils and endothelial cells (28, 29). High plasma levels of MMP-9 have been found in critically ill patients (30), and patients with septic shock (31). We did not find a significant correlation between TNF-α and MMP-9, suggesting that activation of the systemic inflammatory response does not play an important role in the increase of MMP-9 during OLT. The increase in MMP-9 is probably due to a combination of absence of clearance by the liver during the anhepatic period and release from the ischemically injured liver graft after reperfusion. This is substantiated by our observation of sustaining high MMP-9 levels in patients with more severe I/R injury after transplantation. Similar observations regarding such a MMP-9 upregulation have been made in animal models of organ transplantation and I/R injury of liver, heart and lung (32-35).

In contrast with the sharp increase in MMP-9, a decrease in plasma concentrations of MMP-2, TIMP-1 and TIMP-2 was observed. We previously found very high MMP-2 levels in patients with liver cirrhosis (3), while others reported increased levels of TIMP-1 and TIMP-2 in these patients (36, 37), which
were most probably liver tissue-derived and inversely related to liver function. A decline in these parameters, as observed in the present study, could thus be expected. In addition, Cursio et al. demonstrated that TIMP-1 and -2 mRNA do not increase until 24-48 hours after I/R injury (34). Although we observed a peak in MMP-9 levels in the early postreperfusion period, such a peak was not found for the more constitutively expressed MMP-2. This observation is consistent with studies performed by Upadhya et al., who found only small amounts of proMMP-2 and larger amounts of proMMP-9 in the liver graft effluents before reperfusion in patients undergoing OLT (4). These investigators also demonstrated that most organ preservation solutions, used in clinical practice nowadays, contain potent cryptic inhibitors of MMPs (6). The UW preservation solution, which was used in our study, for example, contains lactobionate, which has been shown to be a potent inhibitor of MMPs.

Recently, several interactions between the fibrinolytic and MMP systems have been recognized (2, 11). Plasmin, a key enzyme of the fibrinolytic cascade, may play an important role in the activation of various proMMPs. It acts as a direct activator of proMMP-9 and may be involved in the second step of proMMP-2 activation (11, 13, 38). We have reported that administration of aprotinin, a potent inhibitor of serine-proteases like plasmin, results in a significant reduction of fibrinolytic activity in patients undergoing OLT, exemplified by the prevention of the plasmin-induced increase in D-dimer levels as observed in the placebo group (25). However, we did not find changes in the composition of either MMP-2 or MMP-9 in patients who received regular-dose or high-dose aprotinin, compared with placebo. Furthermore, TIMP-1 and TIMP-2 concentrations were not influenced by the administration of aprotinin. Activation of MMPs during OLT thus appears to occur predominantly via serine-protease-independent mechanisms. Particularly the persistent high MMP-9 levels, with a concurrent general decrease in TIMP-1 and TIMP-2, in the patients with more severe I/R injury might render this protease a valid target for therapeutic intervention. The use of a specific MMP inhibitor has only been studied successfully in a rat model of ischemic liver injury (34), but not yet in human liver transplantation, although speculated to be of potential interest (39).

In summary, this study demonstrates that OLT is associated with a dramatic increase in plasma MMP-9 concentration during the anhepatic and postreperfusion periods. High MMP-9 levels persist in patients with more severe I/R injury, whereas MMP-2, TIMP-1 and TIMP-2 levels gradually decrease throughout the procedure. Our data suggest that MMP-9 upregulation coincides with the development of a generalized ‘lytic’ state during OLT, which has previously been attributed to the activation of other proteases, such as plasminogen activators. Administration of the serine-protease inhibitor aprotinin had no effect on plasma concentrations and composition of MMP-9 or MMP-2, suggesting that enzymes like plasmin are not involved in the activation of proMMPs during OLT.

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Abbreviations

AST, aspartate aminotransferase; BIA, biochemical immunosorbent activity assay; ELISA, enzyme-linked immunosorbent assay; I/R, ischemia and reperfusion; KIU, kallikrein inhibiting units; MMP, matrix metalloproteinase; MT, membrane-type; OLT, orthotopic liver transplantation; TIMP, tissue inhibitor of metalloproteinases; TNF, tumor necrosis factor; t-PA, tissue-type plasminogen activator; UW, University of Wisconsin.

References


