Matrix metalloproteases and their tissue inhibitor in cardiac transplantation

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Abstract

Objective: Multiple studies have shown that matrix metalloproteases (MMPs) are involved in the pathologic reactions occurring as a consequence of cardiac transplantation, including ischemia—reperfusion injury and allograft rejection. This study sought to determine the temporal profile of MMP serum levels following cardiac transplantation.

Methods: Endomyocardial biopsies and serum samples were obtained from 66 recipients at 1, 2, 3, 4, 7, 12, 24, and 52 weeks post-transplant during the routine follow-up protocol, and MMP-1, MMP-8, MMP-9, and tissue inhibitor of metalloproteases (TIMP)-1 serum concentrations were measured by enzyme-linked immunosorbent assay (ELISA). Immunosuppression comprised cyclosporine A (CyA; n = 46) or tacrolimus (TAC; n = 20) with mycophenolate mofetil and steroids.

Results: Increased MMP-8, MMP-9, and TIMP-1 serum levels were observed during the first 2 weeks following transplantation compared to the later time points. MMP-1 was increased at 2 and 3 weeks post-transplant compared to all later time points. No correlation of MMP or TIMP serum concentrations with infection episodes was observed.

Conclusions: Early increase in MMP and TIMP serum levels following cardiac transplantation indicates involvement of these molecules in the reaction of the transplant to ischemia—reperfusion or early immunologic adaptation processes of the host. Further investigation of the relationship between MMP and TIMP serum levels and clinical conditions following transplantation including allograft rejection and hemodynamic graft function is necessary.

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Keywords: Cardiac transplantation; Matrix metalloproteases

1. Introduction

Matrix metalloproteases (MMPs) are involved in extracellular matrix remodeling and immunological processes in various physiological as well as pathological settings [1—3]. In solid organ transplantation, multiple studies have shown that MMPs are involved in allograft tissue injury, including ischemia—reperfusion injury [4] and acute or chronic rejection [5,6]. The function of MMPs in this setting is the degradation of basement membranes that allows influx of leukocytes and mononuclear cells into the graft. In particular, the collagenases MMP-1 and MMP-8 that degrade collagen types I and III and the gelatinase MMP-9 have been implicated in immunological processes [3,7,8]. Additionally, MMP-9 that degrades collagen type I and IV plays a role in ischemia—reperfusion injury [4]. While MMP-8 is primarily produced by neutrophils, MMP-1 and MMP-9 may be secreted by a variety of cell types, including endothelial cells, monocytes/macrophages, lymphocytes, and smooth muscle cells. The activity of MMPs in vivo is tightly regulated by the tissue inhibitors of metalloproteases (TIMPs) [9]. It is known that MMP-8 and MMP-9 cardiac tissue expression levels increase during the first 2 weeks following cardiac transplantation [10]. However, little is known about MMP and TIMP serum levels following cardiac transplantation [11], and the potential clinical applicability of these parameters in the setting of rejection or infection has not been established. To examine the temporal profile of circulating MMPs and TIMPs following heart transplantation, serum MMP-1, MMP-8, MMP-9, and TIMP-1 were measured during the first post-transplant year.

2. Patients and methods

2.1. Patients

Sixty-six consecutive heart failure patients undergoing cardiac transplantation at the Department of Cardiothoracic
Surgery at the Medical University of Vienna between 2002 and 2004, who survived at least 6 months following transplantation, participated in this study. Patients gave informed consent to be enrolled, and the study was approved by the institutional Ethics Committee. All patients were in NYHA class III or IV, matched the American Heart Association guidelines for cardiac transplantation [12], and had optimized pre-transplant heart failure treatment. Patient demographics are shown in Table 1.

Endomyocardial biopsies and serum samples were obtained during routine follow-up at 1, 2, 3, 4, 7, 12, 24, and 52 weeks post-transplant. Endomyocardial biopsies from the right ventricular septum or apex were obtained through a percutaneous right internal jugular vein approach. Serum samples were obtained from the same venous catheter. Serum samples were used for MMP and TIMP measurements by enzyme-linked immunosorbent assay (ELISA) as described below. Additionally, the surveillance protocol included physical status, echocardiography, and serologic tests [13]. Rejection was diagnosed histologically according to the International Society for Heart and Lung Transplantation (ISHLT) guidelines, and infection episodes were assessed as described below.

2.2. Immunosuppression

Immunosuppression comprised cyclosporine A (CyA; \(n = 46\)) or tacrolimus (Tac; \(n = 20\)) with mycophenolate mofetil and steroids. All patients received 500 mg methylprednisolone intra-operatively, and 125 mg at 8, 16, and 24 h post-transplant intravenously. Starting on the first post-operative day patients received antibody induction therapy with polyclonal anti-thymocyte globulin (Thymoglobulin, Cambridge, MA) at 2 mg/kg for 3–7 days (depending on platelet count). Cyclosporine A (Sandimmun Neoral, Novartis, Basel, Switzerland) or tacrolimus (Prograf, Astellas Pharma, Deerfield, IL) was started 3–7 days following transplantation, with target serum levels of 200–250 ng/ml for cyclosporine and 12–15 ng/ml for tacrolimus. Mycophenolate mofetil (Cell Cept, Hofmann-La Roche, Grenzach-Wyhlen, Germany) at 1.5–3 g/day (depending on leucocyte count) was given intravenously on the first postoperative day, and then orally. Prednisolon was given orally at alternating daily doses of 5 and 15 mg [13].

2.3. Diagnosis and prevention of infection

Episodes of infection were identified clinically and approved using microbiologic, serologic, or histologic tests. This included bacterial and fungal cultures in biopsies or in blood, as indicated. Cytomegalovirus (CMV) screening was performed using serologic tests (complementary binding reaction) and measurements of CMV early antigen, as well as PCR for CMV in blood [13]. Patients received prophylaxis for CMV by administration of anti-CMV IgG hyperimmunoglobulin (Cytotect, Biotech, Dreieich, Germany) at 100 ml intravenously immediately following transplantation and on postoperative days 7, 14, and 21. Patients with CMV mismatch and patients with CMV serologic status positive/donor positive were given antiviral prophylaxis using intravenous ganciclovir (Cymevene, Roche, Vienna, Austria) at 10 mg/kg/day for 21 days after transplantation, followed by oral treatment with \(3 \times 500–1000\) mg/day for additional 70 days. Patients also received antibacterial prophylaxis using cefazolin (\(3 \times 2\) g/day) and vancomycin i.v. for 5 days and antifungal prophylaxis with nystatin (100 ml/day) until discharge from hospital [13].

2.4. Enzyme-linked immunosorbent assay for MMPs and TIMP-1

ELISA was performed to detect MMP-1, MMP-8, MMP-9, and TIMP-1 in serum samples, using Quantikine kits (R&D Systems, Minneapolis, MN, USA). Assay diluent was added to each well of microplates pre-coated with specific antibodies. Afterwards, standard or serum samples were added to each well. After incubation for 2 h at room temperature and rinsing, 200 \(\mu\)l of specific conjugates (horseradish peroxidase-conjugated specific antibodies) were added, and incubated for 1 or 2 h at room temperature followed by rinsing. Then, 200 \(\mu\)l substrate solution, consisting of equal volumes of hydrogen peroxide and tetramethylbenzidine, was added to each well and incubated for 30 min at room temperature, protected from light. The reaction was stopped by adding 50 \(\mu\)l stop solution. Then the optical density was detected immediately using a microplate reader (Anthos, Salzburg, Austria) at 450 nm.

2.5. Statistical analysis

MMP and TIMP serum level measurements were compared between time points following transplantation by analysis of variance (ANOVA with Bonferroni correction). Data are expressed as mean ± standard deviation and statistical significance was set to \(P < 0.05\). SAS version 9.1.3 was used for data analysis.

3. Results

All measured MMP and TIMP serum concentrations tended to increase during the first 2 or 3 weeks following heart transplantation, and decreased thereafter. MMP-1 serum concentrations (Fig. 1A) were significantly increased at 2 and 3 weeks post-transplant compared to all later time points \((P < 0.05)\). MMP-8 serum levels were significantly higher at 1 and 2 weeks following cardiac transplantation compared to 3

<table>
<thead>
<tr>
<th>Table 1: Patient demographic characteristics</th>
<th>Ischemic heart failure ((n = 26))</th>
<th>Dilated heart failure ((n = 31))</th>
<th>Other diseases ((n = 9))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (years)</td>
<td>57.5 ± 6.1</td>
<td>50.4 ± 10.7</td>
<td>46.4 ± 15.8</td>
</tr>
<tr>
<td>Male/female</td>
<td>21/5</td>
<td>26/5</td>
<td>5/4</td>
</tr>
<tr>
<td>Ischemia time, mean (min)</td>
<td>167 ± 42</td>
<td>178 ± 49</td>
<td>166 ± 48</td>
</tr>
</tbody>
</table>

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weeks post-transplant or later ($P < 0.05$; Fig. 1B). MMP-9 serum levels were significantly elevated at 1 week following transplantation versus 24 and 52 weeks post-transplant ($P = 0.041$ and $P = 0.035$, respectively; Fig. 1C). TIMP-1 serum levels were significantly increased at 1 and 2 weeks following transplantation compared to the serum levels at 3 weeks post-transplant or later ($P < 0.05$; Fig. 1D). MMP-1 ($r = -0.46; P = 0.0006$), MMP-8 ($r = -0.34; P = 0.023$), and TIMP-1 ($r = -0.76; P < 0.0001$) serum levels were significantly and negatively correlated with time from transplant. MMP and TIMP serum levels were not significantly correlated with age, gender, or ischemia time. In addition, MMP and TIMP serum levels were not significantly associated with C-reactive protein levels or leukocyte cell counts. Rejection episodes occurred in 30 of 66 patients, but none of these patients experienced histological high-grade acute rejection (ISHLT grade 3R). Most rejection episodes were observed at 2, 3, and 4 weeks post-transplant (in 11 patients at 2 weeks, in 13 patients at 3 weeks, and in 10 patients at 4 weeks from transplant). No correlation of MMP or TIMP serum concentrations with infection episodes was observed.

4. Discussion

MMPs and TIMPs are involved in two major processes associated with allograft damage following heart transplantation. The first insult to the donor heart is ischemia—reperfusion injury. This study has shown that MMP-1, MMP-8, MMP-9, and TIMP-1 serum levels increase at 1 or 2 weeks following transplantation and decrease rapidly thereafter. This early increase may be associated with tissue injury and wound healing and the resolving of ischemia—reperfusion injury in the transplanted heart [4]. Additionally, it is known that circulating MMP levels increase after cardiac surgery on cardio-pulmonary bypass [14,15]. For MMP-8 and MMP-9 this pattern matches the results of a study investigating tissue

MMP and TIMP expression following heart transplantation [10]. MMP-1 and TIMP-1 serum levels were negatively correlated to time after transplant in this study, whereas tissue expression of these molecules increased starting at 1 year following transplantation in the tissue expression study [10]. This may be due to different observation periods compared to the study by Schupp et al. [10], which also examined MMP and TIMP expression at 2 and 3 years post-transplant. The second mechanism in which MMPs may be involved in allograft damage following cardiac transplantation regards the immunologic response of the host which may result in acute and chronic graft rejection. In particular, MMP-1 may be involved in the reaction of the recipient to the transplanted heart, because most rejection episodes occurred at the time points at which MMP-1 serum levels were increased, i.e. 2 and 3 weeks following transplantation. This suggests that MMP-1 upregulation may correspond to the initial immunologic activation following transplantation. In line with this, it is known that the T-cell costimulatory factor CD40L induces MMP-1 expression in CD40-expressing cells [16]. MMP-8, MMP-9, and TIMP-1 may also be induced by immunologic processes [3,5,7,8], in addition to their role in ischemia—reperfusion injury [4]. Therefore, the potential correlation between MMP serum levels and clinical conditions following cardiac transplantation, such as allograft rejection, calls for further studies in clinical trials.

References


