Matrix Metalloproteinase-9 and Graft Preservation Injury in Clinical Renal Transplantation

A.J. Turunen[a], L. Lindgren[b], K.T. Salmela[c], L.E. Kyllönen[c], S. Andersson[d], and E. Pesonen[e,*]

[a]Department of Surgery, Kanta-Häme Central Hospital, Hämeenlinna, Finland; [b]Department of Anesthesiology, Tampere University Hospital, Tampere, Finland; [c]Department of Transplantation and Liver Surgery, Helsinki University Hospital, Helsinki, Finland; [d]Children's Hospital, Helsinki University Hospital, Helsinki, Finland; and [e]Department of Anesthesiology and Intensive Care Medicine, Helsinki University Hospital, Helsinki, Finland

ABSTRACT

Background. Deleterious effects of matrix metalloproteinase-9 (MMP-9) have been established in experimental renal ischemia-reperfusion models but not in clinical renal transplantation thus far.

Methods. We studied MMP-9 and its physiological inhibitor tissue inhibitor of matrix metalloproteinases-1 (TIMP-1) in 45 consecutive patients of a larger trial in renal transplantation: perioperative anti-thymocyte globulin (group A, n = 15), perioperative basiliximab (group B, n = 16), and conventional triple therapy (group C, n = 14). In addition to systemic blood samples, local blood samples were obtained simultaneously at 1 and 5 minutes after reperfusion from iliac artery and graft vein for calculation of transrenal changes. Because anti-thymocyte globulin activates inflammation, group A was analyzed separately. Groups B and C were pooled (group BC).

Results. Anti-thymocyte globulin infusion caused a robust rise of MMP-9 in the systemic circulation in group A. No significant transrenal difference of MMP-9 or TIMP-1 occurred in either group during graft reperfusion. In group BC, strong transrenal release of MMP-9 at 1 minute after reperfusion correlated with cold ischemia time ($R = 0.66$, $P = .0001$) and was associated with delayed graft function ($P = .052$).

Conclusions. Renal production of MMP-9 on graft reperfusion is associated with cold ischemia time and emergence of delayed graft function. MMP inhibition may offer a means to reduce reperfusion injury in renal transplantation.

MATRIX METALLOPROTEINASES (MMPs) are a family of zinc-dependent endopeptidases that have been recognized for their ability to degrade components of the extracellular matrix [1]. MMPs have been extensively investigated because they participate in multitude of processes, both physiological and pathological. Among others, they have roles in extracellular matrix homeostasis, spread of malignant tumors and metastases, migration of inflammatory cells, and control of inflammatory vascular permeability.

In solid-organ transplantation, acute inflammatory reactions including responses by neutrophilic white cells are essential in initiation and propagation of deleterious ischemia-reperfusion injury [2]. Neutrophils contain a readily available source of MMP-9 in their tertiary granules that hold this enzyme in significant quantities [3]. Renal MMP-9 has been shown to increase during experimental renal ischemia [4–8]. By destroying the extracellular matrix, MMP-9 may increase microvascular leakage and further enhance extravasation of neutrophils during renal reperfusion injury [8]. Indeed, MMP inhibition confers protection from experimental renal ischemia/reperfusion injury [8–12].

In kidney transplantation, MMP-9 expression is increased in acute rejection [13–15]. Conflicting results have been

This work was supported by EVO grants of TYH2014116 from Helsinki University Hospital.

*Address correspondence to Eero Pesonen, Department of Anesthesiology and Intensive Care Medicine, Meilahti Hospital, University Hospital of Helsinki, FIN-00029 HUS, Helsinki, Finland. E-mail: eero.pesonen@hus.fi

0041-1345/15
http://dx.doi.org/10.1016/j.transproceed.2015.10.056

reported in chronic allograft nephropathy [16–18]. To the best of our knowledge, the role of MMP-9 in graft preservation injury has not been studied thus far in clinical kidney transplantation. In the present study, we investigated MMP-9 and its physiological inhibitor, tissue inhibitor of matrix metalloproteinase 1 (TIMP-1), during clinical renal transplantation with a special focus on the changes in the graft circulation on early reperfusion.

METHODS

Patients for this study were recruited consecutively as a subset of patients who had already agreed to participate in a larger clinical intervention study comparing 3 different regimens of immunosuppression in cadaveric renal transplantation [19]: group A received perioperative 9 mg/kg infusion of anti-thymocyte globulin (ATG) complemented with regular triple-immunosuppression therapy of cyclosporine, azathioprine, and steroids; group B received 2 doses of the interlukin-2 receptor antagonist basiliximab (Simulect, Novartis Pharma AG, Basel, Switzerland) with low initial (5 mg/kg/day) cyclosporine triple therapy; group C received traditional triple immunosuppression with cyclosporine, azathioprine, and steroids. The immunosuppression induction drugs were administered prior to completion of vascular anastomoses, that is, before reperfusion of the kidney graft. This study was integrated to the ongoing intervention study so that a total of 45 consecutive patients were recruited retaining original random assignment: group A, 15 patients; group B, 16 patients; group C, 14 patients.

All grafts were harvested from brain-dead, heart-beating donors through the use of multi-organ procurement techniques. Donor ages did not differ between groups. Grafts were preserved in University of Wisconsin solution at +4°C. The recipient operation was performed under standardized balanced inhalation anesthesia. The surgical technique of the recipient operation was as described [20], with preferably an end-to-end arterial anastomosis to the internal iliac artery and with an open ureteroneocystostomy.

From every patient, central venous blood samples were drawn preoperatively and before reperfusion as well as at 1 and 5 minutes after reperfusion. Preoperative samples were obtained after induction of anesthesia but before surgery. Pre-reperfusion samples were obtained after completion of the vessel anastomoses before de-clamping of the iliac artery and proximal iliac vein. In addition to central venous samples, paired local reperfusion blood samples were obtained at 1 and 5 minutes after reperfusion from the graft supplying artery and vein, that is, from the recipient's iliac artery proximal to anastomosis, and from the renal vein of the graft, respectively. These samples were obtained by direct intraluminal needle aspiration by operating and assisting surgeons before the de-clamping of the distal iliac vein.

All blood samples were drawn into pyrogen-free, plastic disposable syringes and immediately transferred into a glass citrate tube (Vacutainer; Becton Dickinson, Franklin Lakes, NJ, United States) and kept in a Styrofoam box containing 0°C water and ice until centrifugation and plasma separation. Commercial enzyme-linked immunosorbent assay kits measuring plasma MMP-9 and TIMP-1 concentrations were used according to the manufacturers' instructions (Biotrak, Amersham Pharmacia Biotech, Little Chalont, United Kingdom). A 1-mL aliquot was transferred into a glass EDTA tube (Vacutainer) and kept at room temperature until analysis of leukocyte differential counts by use of the Technicon H2 hematology analyzer (Bayer Corporation, Tarrytown, NY, United States). Data concerning leukocyte counts have been previously published in detail [2].

Delayed graft function (DGF) was defined as described by Halloran et al. [21]: plasma creatinine concentration higher than 500 mmol/L throughout the first post-transplant week, or the need of more than 1 dialysis session in the first week, or oliguria of less than 1 L/24 hours for more than 2 days.

Concerning the same study population, unlike in groups B and C, we have previously reported profound activation of both inflammation [2] and coagulation [22] with administration of high-dose ATG in group A. Correspondingly, in the present study, group A differed substantially from groups B and C, whereas groups B and C behaved similarly. Being comparable, if specifically indicated, groups B and C were combined together as group BC in statistical analysis. Statistics are therefore calculated and reported separately for groups A (n = 15), B (n = 16), and C (n = 14), as well as, if specifically indicated, pooled group BC (n = 30). All data were analyzed with the use of SPSS software (SPSS, Inc, Chicago, Ill, United States). Blood samples obtained from the central venous cannula were used for measuring time-dependent changes. Transrenal difference (Δ) for blood sample parameters was calculated as follows: the value of the arterial sample (ingoing) was subtracted from the value of the venous sample (outcoming). The nonparametric Wilcoxon test for paired samples was used for testing significance of transrenal changes of study parameters or testing between 2 time points. The Mann-Whitney *U* test was used for testing of parameter difference between groups. Pearson's correlation was used for correlation analysis. Receiver operating characteristic (ROC) analysis was used for testing of the predictive value of a parameter. Patient data are presented as median and range in the text and as box plots in the figures.

The amendment of the intervention study plan, permitting collection of extra samples for this reperfusion study, was approved by the local ethics committee. Informed consent was obtained from the patients before participating in the study.

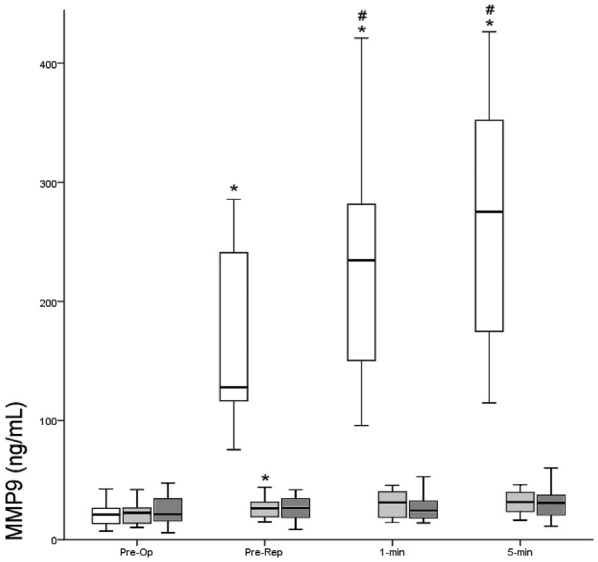

Fig 1. Systemic levels of MMP-9 in groups A (white), B (light gray), and C (dark gray) at different time points: Pre-Op, before surgery; Pre-Rep, immediately before reperfusion; 1-min, 1 minute after vascular de-clamping; 5-min, 5 minutes after vascular de-clamping. *$P < .05$ versus Pre-Op, $P < .05$ versus Pre-Rep.