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Graft Neutrophil Sequestration and Concomitant Tissue Plasminogen Activator Release During Reperfusion in Clinical Kidney Transplantation

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ABSTRACT

Background. Inflammation, coagulation, and fibrinolysis are tightly linked together. Reperfusion after transient ischemia activates both neutrophils, coagulation, and fibrinolysis. Experimental data suggest that tissue plasminogen activator (tPA) regulates renal neutrophil influx in kidney ischemia and reperfusion injury.

Methods. In 30 patients undergoing kidney transplantation, we measured renal neutrophil sequestration and tPA release from blood samples drawn from the supplying artery and renal vein early after reperfusion. tPA antigen levels were measured using a commercial enzyme-linked immunosorbent assay kit. For each parameter, transrenal difference (Δ) was calculated by subtracting the value of the arterial sample (ingoing blood) from the value of the venous sample (outgoing blood).

Results. Positive transrenal gradients of tPA antigen occurred at 1 minute [$\Delta = 14$ (3–46) ng/mL, $P < .01$] and 5 minutes [$\Delta = 5$ (-3 to 27) ng/mL, $P < .01$] after reperfusion. At 5 minutes after reperfusion, a negative transrenal gradient of neutrophils was observed [$\Delta = -0.17$ (-1.45 to 0.24) $\times 10^9$ cells/L, $P < .001$]. At 1 minute after reperfusion, neutrophil sequestration into the kidney (ie, negative transrenal neutrophil count) correlated significantly with tPA release from the kidney (ie, positive transrenal tPA concentration), ($R = -0.513$ and $P = .006$).

Conclusions. The findings suggest a proinflammatory role for tPA in ischemia and reperfusion injury in human kidney transplantation.

INFLAMMATION and coagulation are tightly linked processes [1]. Inflammation drives coagulation through various molecular mechanisms that ultimately lead to enhanced tissue factor expression and thus activation of coagulation cascades. Similarly, several factors in the coagulation and fibrinolytic pathways can trigger either proinflammatory or anti-inflammatory host responses.

Reperfusion after transient ischemia results in activation of both inflammation and coagulation. Thus, surgical ischemia and reperfusion injury (IR-injury) offers a time-controlled clinical model to study the cross talk between inflammatory and coagulation pathways in a localized

environment. We have previously demonstrated the anti-inflammatory properties of activated protein C in clinical IR-injury [2–4].

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Tissue plasminogen activator (tPA) catalyzes conversion of plasminogen to plasmin and thereby enhances fibrinolysis. tPA thus has an integral role in limiting thrombus formation and ensuring blood vessel patency. Beyond its role in fibrinolysis, tPA has direct immunomodulatory functions [5]. In experimental models of IR-injury, tPA regulates tissue neutrophil influx [6–8]. In our kidney transplantation studies, we obtained data on both reperfusion-induced graft neutrophil entrapment [9] and tPA release [10] in the same patient series. Motivated by the above-mentioned experimental reports, we reanalyzed our data to seek evidence for the role of tPA in neutrophil trafficking in vivo in humans.

MATERIALS AND METHODS

The present work is based on 2 previous separate and thorough publications on neutrophil activation [9] and coagulation [10] in clinical kidney transplantation. Briefly, the patient series was a subset of a larger study [11] comparing 3 different regimens of immunosuppression in renal transplantation with deceased donors: anti-thymocyte globulin (ATG, Fresenius AG, Bad Homburg, Germany, group A, $n = 15$), interleukin 2 receptor antagonist basiliximab (Simulect, Novartis Pharma AG, Basel, Switzerland, group B, $n = 16$) and conventional triple immunosuppression with cyclosporine, azathioprine, and steroids (group C, $n = 14$). Group A was omitted because of the strong neutrophil and coagulation and fibrinolysis activating properties of antithymocyte globulin [9,10]. Groups B and C were pooled together ($n = 30$) for final analysis.

Paired blood samples were obtained at 1 minute and 5 minutes after reperfusion from graft-supplying artery and vein (ie, from recipient's iliac artery proximal to anastomosis and from the renal vein of the graft, respectively). Transrenal samples were available in 27 patients at 1 minute and in 28 patients at 5 minutes. A 1-mL aliquot was kept in an EDTA tube (Vacutainer, Becton Dickinson, Franklin Lakes, NJ, USA) in room temperature until leukocyte counts were analyzed by ADVIA 120 hematology analyzer (Bayer Corporation, Tarrytown, NY, United States), and a 4-mL aliquot was kept in a prechilled sodium citrate tube (Vacutainer, Becton Dickinson, Franklin Lakes, NJ, United States) and kept in an ice-water bath (0°C) until plasma was separated for analysis of tPA antigen using a commercial enzyme-linked immunosorbent assay kit (t-PA Actibind, Technoclone, Vienna, Austria).

Transrenal difference (Δ) was calculated for each parameter by subtracting the value of the arterial sample (ingoing blood) from the value of the venous sample (outgoing blood), and the statistical significance was tested with the nonparametric Wilcoxon and Mann-Whitney U tests. Correlations were assessed with Pearson correlation. Categorical data were tested with χ^2 or Fisher exact test, as appropriate. Data are presented as median and range.

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

RESULTS

All grafts were from deceased heart-beating donors, of whom 17 were men and 13 women. The donor age was 39 (14–62) years. The graft cold ischemic time was 22.4

(13.9–35.5) hours. The age of the recipients (14 men and 16 women) was 45 (26–64) years. The transplantation indications were as follows: diabetic nephropathy in 9 patients, polycystic kidney disease in 6 patients, and other indication in 15 patients. Nine patients presented with delayed graft function. Serum creatinine at 3 days postoperatively was 114 (69–475) $\mu\text{mol/L}$ and at 3 months postoperatively 118 (73–178) $\mu\text{mol/L}$.

Significant positive transrenal gradients of tPA antigen were observed both at 1 minute [$\Delta = 14$ (3–46) ng/mL, $P < .01$] and 5 minutes [$\Delta = 5$ (–3 to 27) ng/mL, $P < .01$] after reperfusion [10].

At 1 minute after reperfusion, neutrophil counts did not change transrenally [$\Delta = 0.005$ (–0.75 to 0.42) $\times 10^9$ cells/L], whereas there was marginal but statistically significant negative transrenal gradient of the monocyte count [$\Delta = 0.02$ (–0.1 to 0.2) $\times 10^9$ cells/L, $P = .04$]. At 5 minutes after reperfusion, negative transrenal gradients of neutrophil [$\Delta = -0.17$ (–1.45 to 0.24) $\times 10^9$ cells/L, $P < .001$] and monocyte counts [$\Delta = -0.03$ (–0.1 to 0.06) $\times 10^9$ cells/L, $P = .001$] were observed [9]. When the patients were stratified according to the direction of the transrenal neutrophil gradient (ie, negative or positive), there were no statistically significant differences in donor, recipient, operative, or outcome data between the stratified patients either at 1 minute or 5 minutes after reperfusion (Table 1). Likewise, no differences were observed if the patients were stratified according to the transrenal monocyte gradient (data not shown).

At 1 minute after reperfusion, there was a significant association ($R = -0.513$; $P = .006$) between renal neutrophil sequestration (ie, negative transrenal neutrophil count) and renal release of tPA (ie, positive transrenal tPA concentration) (Fig 1). There were no correlations between tPA and monocyte counts (data not shown).

Transrenal tPA gradient was not associated with postoperative creatinine levels or delayed graft function (data not shown).

DISCUSSION

Experimental data suggest an active pathophysiological role for tPA in IR-injury [6–8]. Although tPA itself is not chemotactant to neutrophils [6,7], endogenous tPA selectively promotes postischemic tissue neutrophil recruitment early after reperfusion [6–8]. Kidneys from tPA knockout mice demonstrated significantly less influx of neutrophils after ischemia when compared to kidneys of wild-type mice [6]. Further, prophylactic tPA-antisense oligonucleotide treatment reduced renal dysfunction, tubular damage, and neutrophil influx of similarly challenged kidneys of wild-type mice [6].

In the present study, high intragraft release of tPA was associated with kidney neutrophil sequestration. There are at least 3 possible explanations to the present finding. Firstly, in clinical thrombolytic therapy, recombinant tPA activates the complement system [12]. Further, tPA

Table 1. Clinical Characteristics and Transrenal Neutrophil Gradient During Reperfusion

	-Δ PMN, 1 Min	+Δ PMN, 1 Min	P Value*, 1 Min	-Δ PMN, 5 Min	+Δ PMN, 5 Min	P Value*, 5 Min
Donor sex, male/female, n (%)	8 (62)/5 (38)	7 (50)/7 (50)	.500	10 (50)/10 (50)	6 (75)/2 (25)	.218
Donor age, y	42 (14–62)	43 (18–62)	.687	43 (14–62)	39 (22–62)	1.000
Donor cause of death, trauma/stroke & hemorrhage, n (%)	6 (46)/7 (54)	9 (64)/5 (36)	.428	12 (60)/8 (40)	4 (50)/4 (50)	.472
Cold ischemic time, h	22.8 (15.9–28.4)	22.4 (13.9–35.5)	.390	23.1 (15.9–35.5)	21.6 (13.9–26.4)	.382
Recipient sex, male/female, n (%)	5 (38)/8 (62)	7 (50)/7 (50)	.348	11 (55)/9 (45)	2 (25)/6 (75)	.155
Recipient age, y	39 (26–64)	44 (29–64)	.287	43 (26–64)	47 (29–63)	1.000
Underlying kidney disease, diabetic/polycystic /other, n (%)	4 (31)/3 (23)/6 (46)	3 (21)/3 (21)/8 (58)	.730	5 (25)/4 (20)/11 (55)	3 (38)/2 (24)/3 (38)	.694
Delayed graft function, n (%)	4 (31)	4 (29)	.613	5 (25)	3 (38)	.442
Serum creatinine at 3 days postoperatively, μmol/L	102 (76–475)	111 (69–213)	.894	106 (69–475)	114 (89–225)	.533
Serum creatinine at 3 months postoperatively, μmol/L	117 (83–144)	113 (73–178)	.979	118 (73–178)	106 (88–131)	.533

Continuous data are expressed as median (range).

- Δ PMN denotes negative transrenal neutrophil gradient; + Δ PMN denotes positive transrenal neutrophil gradient.

*P values are for -Δ PMN vs. +Δ PMN (χ^2 or Fisher exact test and Mann-Whitney U test, as appropriate).

facilitates plasminogen activation into plasmin, which then activates C3 and C5 [13]. Thus, abrupt release of tPA may result in increased intragraft concentrations of complement degradation products chemotactic to neutrophils. Secondly, tPA is expressed on activated neutrophils, and tPA may thus aid neutrophil extravasation via its direct proteolytic properties [8]. Thirdly, tPA may increase neutrophil adherence via plasmin production. Plasmin enhances neutrophil adhesion by a conformational modification of CD18 on the neutrophil surface [14]. On the other hand, tPA increases synthesis of platelet activating factor in the endothelial cells, resulting in increased P-selectin expression on the endothelium [15]. In the latter study, increased neutrophil adhesion was promoted also with tPA alone [15]. Furthermore, endogenous tPA regulates the endothelial expression

of platelet-endothelial cell adhesion molecule 1, an adhesion molecule fundamental to neutrophil diapedesis [16].

The action of tPA in reperfusion may be a double-edged sword. It may break down ischemia-induced microthrombi [10] and thereby enhance perfusion. On the other hand, according to the present clinical and previous experimental data [6–8], tPA increases tissue neutrophil infiltration and may thus aggravate IR-injury [9,17]. In addition to directly promoting neutrophil tissue sequestration, tPA extravasated to the perivascular space activates mast cells (present also in the kidney) to release lipid mediators such as platelet activating factor that further attract neutrophils into the post-ischemic tissue [8].

To the best of our knowledge, this report is the first clinical in vivo evidence proposing a proinflammatory role for postischemic tPA release. However, given the post hoc nature of this data analysis, the present results and the clinical implications of the phenomenon should be confirmed in a prospective study in patients undergoing kidney transplantation.

Fig 1. Graft neutrophil infiltration is associated with concomitant graft tPA release at 1 minute after reperfusion. tPA, tissue plasminogen activator.

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