Published in final edited form as:

J Heart Lung Transplant. 2007 May ; 26(5): 494–497.

ALTERATIONS IN THE FIBRINOLYTIC CASCADE POST TRANSPLANT: EVIDENCE OF A BIMODAL EXPRESSION PATTERN¹

Raymond L. Benza^{2,9}, Matthew A. Cavender³, Joseph Barchue⁴, Jose A. Tallaj⁵, Robert C. Bourge⁶, James K. Kirklin⁷, and Christopher S. Coffey⁸

²*School of Medicine, Department of Medicine, Division of Cardiovascular Disease, University of Alabama at Birmingham (UAB), Birmingham, Alabama 35294*

³*School of Medicine, Department of Medicine, Division of Cardiovascular Disease, University of Alabama at Birmingham (UAB), Birmingham, Alabama 35294*

⁴*School of Medicine, Department of Medicine, Division of Cardiovascular Disease, University of Alabama at Birmingham (UAB), Birmingham, Alabama 35294*

⁵*School of Medicine, Department of Medicine, Division of Cardiovascular Disease, University of Alabama at Birmingham (UAB), Birmingham, Alabama 35294*

⁶*School of Medicine, Department of Medicine, Division of Cardiovascular Disease, University of Alabama at Birmingham (UAB), Birmingham, Alabama 35294*

⁷*Department of Surgery, Division of Cardiothoracic Surgery, University of Alabama at Birmingham (UAB), Birmingham, Alabama 35294*

⁸*School of Public Health, Department of Biostatistics University of Alabama at Birmingham (UAB) Birmingham, Alabama 35294*

Abstract

Changes in the fibrinolytic system that occur after cardiac transplantation (CTx) and the factors which influence such changes are poorly described, yet may be ultimately important in determining the varying morphologic features of transplant related coronary artery disease (Tx CAD). Baselines demographic, as well as serial clinical information and plasma fibrinolytic levels were prospectively recorded preCTx and multiple times postCTx in 110 de novo cardiac transplant recipients. We noted a biphasic change in fibrinolytic activity over the first year of CTx with an early immediate decline in PAI-1 activity ($p > 0.001$) matched with stable PAP (plasmin) activity corresponding to an “enhanced” fibrinolytic state early post CTx followed by a significant increase at 6 months ($p = 0.004$) and 1 year ($p < 0.001$) in PAI-1 activity concomitant with a significant decline in PAP after 3 months ($p = 0.005$ at 3 months, $p < 0.001$ at 6 months, and $p < 0.001$ at 1 year) corresponding to an “impaired” fibrinolytic state late post CTx. This biphasic nature of the fibrinolytic system could account for the varying morphologic features of Tx CAD.

¹Supported by NIH grant: K08 HL03789. No conflict of interest.

Corresponding Author: Raymond L. Benza, M.D., University of Alabama at Birmingham, 1900 University Boulevard, THT 321N, Birmingham, AL 35294-0006, rbenza@uab.edu

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Keywords

Cardiac Transplantation; Chronic Rejection; Fibrinolysis; Clinical Transplantation; Allograft Coronary Disease

The development of an accelerated form of coronary artery disease (Tx CAD) significantly limits the long-term success of cardiac transplantation (1,2). Tx CAD is common and detected angiographically in 44-79% of recipients by 5 years (1,3-7) and nearly universal by IVUS by one year (8). Despite the advancements in clinical immunosuppression, the incidence and prevalence of Tx CAD remains constant (3-7,9). Limitations in donor availability and the diminished survival associated with retransplantation (10,11) underscore the need for further insight and research.

The development of Tx CAD particularly intimal proliferation is due to a complicated interplay between immune and nonimmune factors. Among the nonimmune factors, alterations in fibrinolytic activity (FA) appear to be important (12-16). Impaired FA usually results from diminished levels of the plasminogen activators (PAs), t-PA and urokinase (u-PA) and/or the presence of excess PAI-1, the inhibitor of these PAs. This results in decreased plasmin production and fibrin deposition, which is a common feature of grafts with angiographically detectable Tx CAD (atheromatous form). The effects of enhanced fibrinolysis or increased plasmin activity on Tx CAD are less known, but may be important in the development of the early intimal response.

The purposes of this observational study are to characterize the changes in components of the fibrinolytic system in transplant recipients over time and determine whether these changes could feasibly influence the sequential changes in morphology seen typically in Tx CAD.

METHODS

Patients

Between 06/01/1997 and 12/01/2001, 110 denovo cardiac transplants were prospectively enrolled. Informed consent was obtained from all patients. This study was approved by the Institutional Review Board at UAB. Serial plasma t-PA, PAI-1, u-PA, fibrinogen levels, PAI-1 activity, and plasmin/alpha-2-anti-plasmin (PAP) assays (plasmin activity) were recorded preCTx and postCTx (1 week; 1, 3, 6, and 12 months).

Donor and Recipient Demographics

Data extracted at baseline (preCTx) included specific information on donor/recipient age, gender, race, recipient BMI, smoking status, blood pressure (mmHg), and presence of diabetes mellitus. In addition to serial plasma fibrinolytic levels, we collected serial recipient BMIs, blood pressures, creatinine levels, immunosuppressants (dosing/levels), CMV reactivity, rejection episodes, lipoproteins, as well as lipid-lowering and anti-hypertensive drug use.

Statistical Analysis

The percentage change in fibrinolytic protein levels and activity from baseline was computed for each time point. Due to the skewness of the distributions, we applied a logarithmic transformation to all values. We utilized a t-test to test for a significant change from baseline at each time point, using a Bonferroni correction to adjust for multiple comparisons. For those serial levels that were of most interest (PAI-1 activity and PAP), we also fit a repeated measures mixed model to examine change from baseline during the first year postCTx. These mixed models examined linear, quadratic, and cubic trends over time, adjusting for baseline values.

In order to reduce the problem of multi-collinearity often present in polynomial models, we subtracted the integer value closest to the mean values of time for each individual value. The models also utilized a random intercept for each patient to account for the fact that measurements observed over time within a patient were correlated. The models with change in PAP as the outcome also included a random slope for each patient to account for the fact that the variation in measurements tended to increase over time.

We also considered a set of predictors of PAI-1 activity and PAP changes in the first year postCTx consisting of clinical variables (BMI, blood pressure, lipid fractions, creatinine, glucose, diabetes); use of tacrolimus, atorvastatin, pravastatin, ACE inhibitors, cyclosporine, mycophenolate mofetil, OKT3, prednisone; cumulative dosage for ACE inhibitors, cyclosporine, mycophenolate mofetil, prednisone; and the presence of rejection or CMV infection. Due to the fact that this was an exploratory analysis with a small sample size, we made no adjustment for multiple comparisons and used a less stringent significance level of 10% for determining statistical significance. All variables reaching this level of significance in the univariate analysis were entered into a multivariable model using backwards elimination procedures to determine whether the effect remained after adjustment.

RESULTS

PAI-1, t-PA, u-PA, and Fibrinogen Levels

Fibrinogen and t-PA levels were increased slightly after transplant, but the increases were not significant, except for the 3 month value of t-PA ($p < 0.001$). u-PA levels decreased significantly within the first month postCTx ($p = 0.001$) but returned to baseline levels within three months. PAI-1 antigen levels decreased significantly postCTx ($p < 0.001$ one week post) and remained below baseline values for the first year.

PAI-1 Activity and PAP Complexes (Figure 1)

There was an initial significant reduction in PAI-1 activity immediately postCTx ($p < 0.001$ one week post). However, as opposed to PAI-1 antigen levels, PAI-1 activity then began to increase for the remainder of the first year postCTx. As a consequence, there was a significant increase in PAI-1 activity at 6 months ($p = 0.004$) and 1 year ($p < 0.001$) postCTx.

PAP levels, a marker of plasmin activity, were not significantly different from baseline for the first 3 months postCTx. However, from 3 months on, there was a significant reduction in PAP levels ($p = 0.005$ at 3 months, $p < 0.001$ at 6 months, and $p < 0.001$ at 1 year).

Determinants of PAI-1 Activity and PAP Levels Post Transplant (Tables 1 and 2)

By univariate analyses, > 1 rejection episode, a lower ACE inhibitor dose, and a higher cyclosporine (CsA) dose were significantly associated with greater increases in PAI-1 activity over time. In the multivariable model, however, only rejection episodes, and a higher dose of CsA remained independent predictors for increased PAI-1 activity over time (Table 1).

A similar analysis was performed to assess predictors of serial PAP levels. By univariate analyses, a greater L(p)a change from baseline, not taking tacrolimus or mycophenolate mofetil were significantly associated with greater increases over time in PAP levels. In the multivariable model not taking mycophenolate remained an independent predictor (Table 2).

DISCUSSION

Tx CAD is characterized by a unique, overlapping biphasic pattern of vascular remodeling starting with a diffuse intimal proliferation early followed by a more classical and coexistent

atheromatous component (2,17-20). The fibrinolytic system is likely important in both developing patterns. The question we sought to answer was whether changes in FA could provide a plausible mechanism to explain the sequential morphologic changes seen in progressive Tx CAD. This study shows that two phases of FA exists post CTx that may explain these morphologic patterns and that these phases may be influenced by clinical factors unique to the transplant environment.

The first indication of Tx CAD is the formation of the neointima, which is characterized by intense cell movement and proliferation and requires enhanced, not impaired FA. This is the first key piece of information needed to understand and appreciate how the unique biphasic nature of the fibrinolytic system post transplant could lead to the varying morphologic features of Tx CAD. As noted in this study, PAI-1 activity decreases immediately postCTx with a relative preservation of plasmin activity, which suggests that a plasmin-dominated, profibrinolytic state is present in the short period immediately post transplantation. Plasmin is pivotal to the development of the neointima via the proliferation and migration of SMCs (16, 21-23). Plasmin's nonproteolytic properties also promote the neointima by acting as a chemo attractant for macrophages and lymphocytes (24). It appears that the net result of these cellular mechanisms is an adaptive intimal remodeling process that correlates with the intimal proliferation seen during the first months in allograft vessels (8).

The atheromatous phase of Tx CAD occurs later postCTx and takes advantage of and builds upon the already expanding neointima (17-19,25). This phase, however, is propagated by impaired, not enhanced FA marked by heightened PAI-1 activity. This prothrombotic state change during the later part of year 1 correlates well with the microvascular fibrin deposition characteristic of mature Tx CAD (14,15), as well as the increase in angiographically evident Tx CAD by 3 years (20).

Thus, two distinct fibrinolytic states exist postCTx and these sequential changes in FA could theoretically influence the morphologic shift in Tx CAD. Our analysis also found several clinical factors and medications that may be important in predicting overall fibrinolytic and thrombotic activity. Given that some of these same factors also are known to either promote or mitigate both neointimal proliferation and atheromatous CAD, it is possible that these clinical factors could also influence the formation and/or progression of Tx CAD via mechanisms involving shifts in FA (26-34).

This study is limited by the small, single center nature of the cohort and that some time points contain missing values, which could skew the significance of the time course. In addition the independent, albeit transient, effect of cardiopulmonary bypass on the early profibrinolytic state via its effect on t-PA, PAI-1 and plasmin must be considered as a contributor to this process (35,36). In the future, we hope to expand this study to multiple transplant centers in an attempt to solidify the relationship between clinical variables and FA as well as to use changes in FA to predict the development of Tx CAD.

References

1. Costanzo MR, Naftel DC, Pritzker MR, Heilman JK 3, Boehmer JP, Brozena SC, Dec GW, Ventura HO, Kirklin JK, Bourge RC, Miller LW. Heart transplant coronary artery disease detected by coronary angiography: a multiinstitutional study of preoperative donor and recipient risk factors. The Cardiac Transplant Research Database. *J Heart Lung Transplant* 1998;17:744–753. [PubMed: 9730422]
2. Kobashigawa JA, Tobis JM, Starling RC. Multicenter intravascular ultrasound validation study among heart transplant recipients: outcomes after five years. *J Am Coll Cardiol* 2005;3:1532–1537. [PubMed: 15862430]

3. O'Neill BJ, Pflugfelder PW, Singh MR, Menkin AH, McKenzie FN, Kostuk WJ. Frequency of angiographic detection and quantitative assessment of coronary arterial disease one and three years after cardiac transplantation. *Am J Cardiol* 1989;63:1221–1226. [PubMed: 2653018]
4. Mullins PA, Shapiro LM, Aravot DA, Scott JP, Large SR, Wallwork J, Schofield PM. Experience of percutaneous transluminal coronary angioplasty in orthotopic cardiac transplant recipients. *Eur Heart J* 1991;12:1205–1207. [PubMed: 1782950]
5. Balk AH, Simmons ML, vd Linden MJ, de Feyter PJ, Movhtar B, Weimar W, Bos E. Coronary artery disease after heart transplantation: Timing of coronary arteriography. *J Heart Lung Transplant* 1993;12:89–99. [PubMed: 8443207]
6. Gao S-Z, Schroeder JS, Alderman EL, Hunt SA, Valentine HA, Wiederhold V, Stinson EB. Prevalence of accelerated coronary artery disease in heart transplant survivors. Comparison of cyclosporine and azathioprine regimens. *Circulation* 1989;80:100–105.
7. Sandhu JS, Uretsky BF, Reddy PS, Denys BG, Ruffner RJ, Breisblatt WM, Zerbe TR, Kormos RL, Armitage JM, Hardesty RL, et al. Potential limitations of percutaneous transluminal coronary angioplasty in heart transplant recipients. *Am J Cardiol* 1992;69:1234–1237. [PubMed: 1575196]
8. Ventura HO, Ramee SR, Jain A, White CJ, Collins TJ, Mesa JE, Murgu JP. Coronary artery imaging with intravascular ultrasound in patients following cardiac transplantation. *Transplantation* 1992;53:216–219. [PubMed: 1733071]
9. Olivari MT, Holman DC, Wilson RF, Kubo SH, Ring WS. Coronary artery disease in cardiac transplant patients receiving triple-drug immunosuppressive therapy. *Circulation* 1989;80:111–115.
10. Gao SZ, Schroeder JS, Hunt S, Stinson EB. Retransplantation for severe accelerated coronary artery disease in heart transplant recipients. *Am J Cardiol* 1988;876–881. [PubMed: 3052011]
11. Smith JA, Ribakove GH, Hunt SA, Miller J, Stinson EB, Oyer P, et al. Heart retransplantation: the 25-year experience at a single institution. *J Heart Lung Transplant* 1995;14:832–839. [PubMed: 8800717]
12. Benza RL, Grenett HE, Bourge RC, Kirklin JK, Naftel DC, Castro PF, McGiffin DC, George JF, Booyse FM. Gene polymorphisms for PAI-1/t-PA and development of allograft coronary artery disease. *Circulation* 1998;98:2248–2254. [PubMed: 9826310]
13. Benza RL, Anderson PG, Lyle K, Barchue J, de Oliverira AL, Cavender MA, Pinderski LJ, George JF. Donor PAI-1 expression inhibits the intimal response of early allograft vascular disease. *J Heart Lung Transplant* 2003;22:515–518. [PubMed: 12742412]
14. Labarrere CA, Pitts D, Halbrook H, Faulk WP. Tissue plasminogen activator, plasminogen activator inhibitor-1, and fibrin as indexes of clinical course in cardiac allograft recipients: An Immunocytochemical Study. *Circulation* 1994;89:1599–1608. [PubMed: 8149527]
15. Labarrere CA, Nelson DR, Faulk WP. Myocardial fibrin deposits in the first month after transplantation predict subsequent coronary artery disease and graft failure in cardiac allograft recipients. *Am J Med* 1998;105:207–213. [PubMed: 9753023]
16. Moons L, Shi C, Ploplis V, Plow E, Haber E, Collen D, Carmeliet P. Reduced transplant arteriosclerosis in plasminogen-deficient mice. *J Clin Invest* 1998;102:1788–1797. [PubMed: 9819364]
17. Bieber CP, Stinson EB, Shumway NE, Payne R, Kosek J. Cardiac transplantation in man. VII. Cardiac allograft pathology. *Circulation* 1970;41:753–772. [PubMed: 4910523]
18. Johnson DE, Gao SZ, Schroeder JS, DeCampi WM, Billingham ME. The spectrum of coronary artery pathologic findings in human cardiac allografts. *J Heart Transplant* 1989;8:349–359. [PubMed: 2795279]
19. Uys CJ, Rose AG. Pathologic findings in long-term cardiac transplants. *Arch Pathol Lab Med* 1984;108:112–116. [PubMed: 6365025]
20. Kapadia SR, Ziada KM, L'Allier PL, Crowe TD, Rincon G, Hobbs RE, Bott-Silverman C, Young JB, Nissen SE, Tuzcu EM. Intravascular ultrasound imaging after cardiac transplantation: advantage of multi-vessel imaging. *J Heart Lung Transplant* 2000;19:167–172. [PubMed: 10703693]
21. Brodsky S, Chen J, Lee A, Akassoglou K, Norman J, Goligorsky MS. Plasmin-dependent and -independent effects of plasminogen activators and inhibitor-1 on ex vivo angiogenesis. *Am J Physiol Heart Circ Physiol* 2001;281:H1784–H1792. [PubMed: 11557572]

22. Chapman HA. Plasminogen activators, integrins, and the coordinated regulation of cell adhesion and migration. *Curr Opin Cell Biol* 1997;9:714–724. [PubMed: 9330876]
23. Loskutoff DJ, Quigley JP. PAI-1, fibrosis, and the elusive provisional fibrin matrix. *J Clin Invest* 2000;106:1441–1443. [PubMed: 11120750][comment]
24. Ploplis VA, Castellino FJ. Nonfibrinolytic functions of plasminogen. *Methods* 2000;21:103–110. [PubMed: 10816371]
25. Billingham ME. Cardiac transplant atherosclerosis. *Transplant Proc* 1987;19:19–24. [PubMed: 3303565]
26. Shimizu H, Takahashi M, Takeda S, Inoue S, Fujishiro J, Hakamata Y, Kaneko T, Murakami T, Takeuchi K, Takeyoshi I, Morishita Y, Kobayashi E. Mycophenolate mofetil prevents transplant arteriosclerosis by direct inhibition of vascular smooth muscle cell proliferation. *Transplantation* 2004;77:1661–1667. [PubMed: 15201664]
27. Scheller B, Grandt A, Wnendt S, Lorenz G, Bohm M, Nickenig G. Comparative study of tacrolimus and paclitaxel stent coating in the porcine coronary model. *Z Kardiol* 2005;94:445–452. [PubMed: 15997345]
28. Benza RL, Brown RN, Kirklin JK, Hubbard M, Zoghbi GJ, McGiffin DC, Bourge RC, Rayburn BK, Foley BA. Palliation of allograft vasculopathy with transluminal angioplasty - a decade of experience. *J Heart Lung Transplant* 2002;21:74–75.
29. Patrassi GM, Sartori MT, Rigotti P, Di LD, Theodoridis P, Fioretti M, Capalbo M, Saggiorato G, Boeri G, Girolami A. Reduced fibrinolytic potential one year after kidney transplantation. Relationship to long-term steroid treatment. *Transplantation* 1995;59:1416–1420. [PubMed: 7770928]
30. Vathsala A, Weinberg RB, Schoenberg L, Grevel J, Goldstein RA, Van Buren CT, Lewis RM, Kahan BD. Lipid abnormalities in cyclosporine-prednisone-treated renal transplant recipients. *Transplantation* 1989;48:37–43. [PubMed: 2665233]
31. Verpooten GA, Cools FJ, Van der Planken MG, Bedert LC, Claes R, Van Gaal LF, De Broe ME. Elevated plasminogen activator inhibitor levels in cyclosporin-treated renal allograft recipients. *Nephrol Dial Transplant* 1996;11:347–351. [PubMed: 8671791]
32. Yusuf S, Sleight P, Pogue J, Bosch J, Davies R, Dagenais G. Effects of an angiotensin-converting-enzyme inhibitor, ramipril, on cardiovascular events in high-risk patients. The Heart Outcomes Prevention Evaluation Study Investigators. *N Engl J Med* 2000;342:145–153. [PubMed: 10639539]
33. Kato T, Chan MC, Gao SZ, Schroeder JS, Yokota M, Murohara T, Iwase M, Noda A, Hunt SA, Valentine HA. Glucose intolerance, as reflected by hemoglobin A1c level, is associated with the incidence and severity of transplant coronary artery disease. *J Am Coll Cardiol* 2004;43:1034–1041. [PubMed: 15028363]
34. Bastard JP, Pieroni L, Hainque B. Relationship between plasma plasminogen activator inhibitor 1 and insulin resistance. *Diabetes Metab Res Rev* 2000;16:192–201. [PubMed: 10867719]
35. Paparella D, Brister SJ, Buchanan MR. Coagulation disorders of cardiopulmonary bypass: a review. *Intensive Care Med* 2004;30:1873–1881. [PubMed: 15278267]
36. Linden MD. The hemostatic defect of cardiopulmonary bypass. *J Thromb Thrombolysis* 2003;16:129–147. [PubMed: 15087599]

ABBREVIATIONS

UAB	University of Alabama at Birmingham
CTx	Cardiac transplantation
Tx CAD	Transplant-related coronary artery disease
IVUS	Intracoronary ultrasound

MIT	Maximal intimal thickness
FA	Fibrinolytic activity
SMCs	Smooth muscle cells
t-PA	Tissue plasminogen activator
scu-PA	Single chain urokinase type plasminogen activator
PAI-1	Plasminogen activator inhibitor-1
PAs	Plasminogen activators
u-PA	Urokinase plasminogen activator
PAP	Plasmin/alpha-2-anti-plasmin assays

Figure 1.

Table 1

Predictors associated with increased change in percentage of baseline PAI-1 activity over time

Predictors	Correlation	Univariate P-Value	Multivariable P-Value
Indicator of Rejection	Positive	0.04	0.06
ACE Inhibitor Dosage	Negative	0.08	NS
Cyclosporine Dosage	Positive	0.10	0.06

Table 2

Predictors associated with increased change in percentage of baseline PAP levels over time

Predictors	Correlation	Univariate P-Value	Multivariable P-Value
L(p)a Antigen	Positive	0.04	0.10
Insulin Antigen	Negative	0.09	N/A
Indicator of FK506 Usage	Negative	0.07	NS
Indicator of Cellcept Usage	Negative	0.01	0.02