Published in final edited form as:

J Heart Lung Transplant. 2007 December ; 26(12): 1281–1285.

RECOMBINANT ADENOVIRAL GENE TRANSFER DOES NOT AFFECT CARDIAC ALLOGRAFT VASCULOPATHY

Vinay P. Rao¹, Stefano E. Branzoli, MD¹, Davide Ricci, MD^{1,3}, Naoto Miyagi, MD¹, Timothy O'Brien, MD, PhD², Henry D. Tazelaar, MD¹, Stephen J. Russell, MD, PhD², and Christopher G. A. McGregor, MB, FRCS, MD(Hons)¹

¹ Mayo Clinic-Rochester William J von Liebig Transplant Center

² Molecular Medicine Program, Mayo Clinic Rochester, MN 55905 USA

³ Foundation Policlinico San Matteo, University of Pavia, Pavia, Italy

Abstract

Introduction—Adenovirus serotype 5 has remained the preeminent vector in pre-clinical gene therapy applications in cardiac transplantation. Concerns over the potential effects of adenoviral vectors on the later development of cardiac allograft vasculopathy (CAV) were addressed in this study.

Methods—Hearts (n=22) harvested from Brown Norway rats were perfused *ex vivo* with either UW solution with no virus, Ad-CMV-LacZ or Ad-CMV-Null. Donor hearts were transplanted heterotopically into the abdomen of Lewis rats. All recipients received Cyclosporine for the duration of the experiment. Transplanted hearts were recovered for analyses at 120 days. Sections of the heart were stained with elastic Van Gieson stain for morphometric analysis of the vessels to ascertain the degree of vascular luminal occlusion. Haematoxylin-eosin staining facilitated diagnosis of chronic rejection.

Results—77% of transplanted hearts showed signs of chronic rejection with no difference in the proportion of animals between the groups (p=0.797). No difference was noted in the degrees of vascular luminal occlusion between Ad-Null (0.57±0.22), Ad-LacZ (0.62±0.19) and UW groups (0.47±0.29) (p=0.653).

Conclusions—Vascularized cardiac allografts transplanted from Brown Norway to Lewis rats demonstrate the presence of CAV at 120 days. Adenoviral perfusion of the donor heart *ex vivo* does not affect the development of CAV.

Keywords

Gene therapy; Adenovirus; Cardiac allograft vasculopathy; Heart transplantation

Corresponding author: Christopher G. A. McGregor, MB, FRCS, MD(Hons), Professor of Surgery, Mayo Clinic College of Medicine, Mayo Clinic-Rochester William J von Liebig Transplant Center, 200 First Street SW, Rochester, MN 55905 USA, Telephone: 507-255-6038, Fax: 507-255-4500, e-mail: mcgregor.christopher@mayo.edu.

Presented at the 27th Annual Meeting and Scientific Sessions of the International Society for Heart and Lung Transplantation

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.

INTRODUCTION

With an estimated incidence of 50% at 5 years, cardiac allograft vasculopathy (CAV) is the biggest impediment to long-term disease free survival after cardiac transplantation.(1) Limited success with conventional therapy has led investigators to consider viral vector-directed gene therapy approaches to reduce the incidence and progression of this disease.

However, viruses have been implicated in the development of CAV, chiefly through their effects on graft endothelial function.(2) The expression of adenoviral proteins in pediatric allografts demonstrating the presence of transplant vasculopathy(3) and the adverse effects noted with adenoviral infections in immunosuppressed patients(4) have led to concerns over the use of adenoviral vectors in pre-clinical gene therapy applications in cardiac transplantation.(5) Although Adenovirus (Ad) serotype 5-based transduction of genes have shown promise in affecting transplant vasculopathy (6–9) prolonging cardiac allograft survival (10,11) and promoting neovascularization in the ischaemic myocardium(12), adenoviral gene transfer is known to elicit a potent innate and acquired humoral and cellular inflammatory response(13–16) that could trigger changes leading to CAV. Therefore, the effect of the virus on cardiac vasculature needs to be ascertained prior to assessment of the effects of therapeutic genes on graft vasculopathy.

In this study, we attempted to address concerns over the effects of the viral vector on the later development of CAV following *ex vivo* perfusion of the donor heart.

MATERIALS AND METHODS

Animals

Inbred Lewis rats (250–300g, RT1^L) and Brown-Norway rats (250–300g, RT1ⁿ) were used as recipients and donors, respectively, for allogenic abdominal heterotopic heart transplantation. Procedure and handling of animals were reviewed and approved by the Institutional Animal Care and Use Committee of Mayo Clinic and Foundation in compliance with “Principles of Laboratory Animal Care” formulated by the National Society for Medical Research and the “Guide for the Care and Use of Laboratory Animals” prepared by the Institute of Laboratory Animal Resources and Published by the National Institute of Health (National Institute of Health publication No.86-23, revised 1985).

Adenoviral vectors

A replication defective E1a-deleted serotype 5 adenoviruses encoding for non-nuclear targeted Escherichia Coli β -galactosidase under the control of the CMV promoter were used in this study. Ad-Null, an identical vector not containing the β -Galactosidase expression cassette was used to ascertain the effects of the vector independent of the transgene. (Ad-CMVLacZ and Ad-Null, provided by James Wilson, Institute for gene therapy, University of Pennsylvania).

Experimental Groups

Rats were randomly assigned to one of three experimental groups. Hearts were harvested and perfused *ex vivo* using techniques described previously.(17) Briefly, cardioplegia was obtained by infusion of 5 ml of UW solution through the cannula inserted in the innominate artery, the heart was harvested and the pulmonary artery was cannulated with a 14G cannula. With the inflow cannula in the innominate artery and the outflow cannula in the pulmonary artery, 5 ml of UW were recirculated at 4°C for 15 minutes. In group C (control group) (8 animals) hearts were perfused with UW solution as previously described (17) at a flow of 0.75 ml/min. In group A (AdNull group n=6) and B (β -Gal group n=8 animals) hearts were treated as in group C with the difference that in UW were diluted 3.5×10^8 pfu (total) of Ad-Null and Ad-LacZ,

respectively. After the 15 minute-perfusion period, hearts were stored in UW solution at 4°C during recipient preparation.

Recipient operation

Preparation of the recipient and heterotopic abdominal heart transplantation were performed as previously described.(18) All rats received post-operative analgesia. All animals received 5 mg/Kg of cyclosporine A 3 times a week intramuscularly for the duration of the experiment.

Presence of graft coronary disease

After 120 days all rats were sacrificed by deep pentobarbital anesthesia (70 mg/kg) the transplanted hearts were harvested. Mid ventricular sections were fixed in formalin and routinely processed for histopathological analysis. Sections were cut and placed in OCT compound, snap frozen in liquid nitrogen and stored for immunohistochemical analyses.

Two routinely processed sections for each animal were stained with haematoxylin and eosin (H&E) in order to determine the presence of chronic rejection. In order to assess the degree of vessel occlusion slides were stained with elastic Van Gieson to highlight the elastic lamina (EL). Five slides were prepared for each rat, and the occlusion ratio of each vessel able to be visualized was recorded. The degree of intimal hyperplasia was morphometrically assessed by using the formula: luminal occlusion= (Internal elastic laminal area-luminal area)/Internal elastic laminal area.(19) Over 1000 vessels were analyzed for neointimal proliferation with an average of 50 vessels from each animal. Vessels of hearts with no histological evidences of chronic rejection were considered negative controls.

Statistical Analysis

The mean occlusion ratios for each rat were compared using analysis of variance (SAS GLM procedure) to look for an overall difference between the groups. A mixed effects model was used to make comparisons between pairs of groups using every measurement for every rat, treating individual rats as random effects. The proportion of animals with evidence of rejection in each treatment group was compared using Fisher's exact test. All data are expressed as mean $\pm$ SD. A *P* value of <0.05 was consider significant.

RESULTS

All animals completed the protocol

17 of 22 (77%) transplanted hearts showed evidence of chronic rejection on H&E stained slides. No difference was noted between the groups in the proportion of animals showing evidence of chronic rejection (UW 3/8, Ad-Null 1/6 and AdLacZ 1/8, $p=0.797$). The average number of vessels visualized per rat were 49.4 ± 13.2 in the UW group, 39.2 ± 10.3 in the Ad-Null group and 60.1 ± 8.1 in the Ad-LacZ group. Specimens from rats in UW-treated group had fewer measurable vessels than from rats in other groups ($p=0.017$) Mean occlusion ratios in the groups were as follows: Ad-Null (0.57 ± 0.22), AdLacZ (0.62 ± 0.19) and UW groups (0.47 ± 0.29) Comparison of the means occlusion ratios between the groups did not reveal any significant difference ($p=0.511$). (Figures 1a,b and 2)

DISCUSSION

These results confirm that vascularized cardiac grafts from Brown Norway to immunosuppressed Lewis rats demonstrate the presence of CAV at 4 months. *Ex vivo* perfusion of donor hearts with either the adenovirus serotype 5 expressing LacZ or Ad-Null at the time of transplant did not affect the development of graft vasculopathy.

The pathophysiology of cardiac allograft vasculopathy has yet to be fully understood. Leading hypotheses suggest that incompletely suppressed immune and non-immune factors initiate cytokine and growth factors cascades that evoke endothelial injury leading to neointimal hyperplasia and VSMC proliferation.(20–22) Viral vectors induce a potent host innate and adaptive immune response through the activation of chemokines(23), and by up-regulating endothelial adhesion molecules and promoting T-cell infiltration at the sites of gene transfer, could provoke endothelial injury and stimulate graft vasculopathy. This effect was not confirmed in our comparisons of hearts perfused with Ad-Null with those perfused with UW solution. In addition, we considered the possibility that the transplanted organ could face a specific β -galactosidase reporter gene -induced adaptive immune response that could impact the later development of graft vasculopathy. However, a lack of such a difference between coronary artery occlusion ratios between Ad-null perfused hearts and LacZ-perfused hearts did not justify this concern.

In contrast to previous studies (15) reporting a positive correlation of adenoviral transduction with the development of intimal hyperplasia, we used a lower dose of the vector and perfused the heart at 4° C *ex vivo*. Studies using *ex vivo* perfusion of the heart under hypothermic conditions have revealed an absent(24) or mild(25) inflammatory response. It has also been suggested that an inflammatory response following adenoviral transfection is organ specific and that the low antigen-presenting cell (APC) content of the heart might favour long-term gene expression(26). It is possible that suppression of inflammation due to adenoviral infection with CsA prevents the acceleration of CAV although the development of CAV in the face of anti-T cell therapy in this study simulates the clinical situation more closely (27).

It is interesting that fewer vessels were noted in the UW-perfused hearts and the possibility of inflammation-induced angiogenesis following effective adenoviral transduction was considered. In pediatric hearts free of atherosclerosis, inflammation and adventitial angiogenesis are associated with CAV (28). However, an acute inflammatory response following replication-defective Ad transduction is unlikely to lead to an angiogenic response months following transplantation. In addition, the Ad-perfused organs did not demonstrate a higher incidence of graft vasculopathy suggesting the need for further scrutiny of the precise mechanisms underlying this association.

Although the profile or distribution of transfected cells in the heart or the efficiency of gene transduction have not been defined in this study, our previous experience with this perfusion technique has demonstrated a gene transduction efficacy of 1.8% in the subendocardial region to 45 % in the subepicardial region, with the transgene showing a definite preference to the cardiomyocyte over the vascular endothelium.(24,25) More recent studies(29) have reported functional effects of adenoviral gene transfer using a similar hypothermic *ex vivo* perfusion technique emphasizing the importance of assessing qualitative rather than quantitative end points of gene transfer. The hypothermic *ex vivo* perfusion technique has been validated by several groups (17,30,31) reporting reliable and consistent transduction of the reporter gene. Longitudinal examination of short-term adenoviral gene transduction as well as its effects on CAV in the same animal is not possible in this model although it could be feasible with the use of reporter genes that allow non-invasive imaging.

Recent studies investigating the molecular mechanisms of CAV by microarray analysis of gene expression profiles present new molecular targets for a gene-therapy approach to disease (32). Although short-term expression of first-generation adenoviral vector-based genes limit their use to attenuation of processes like ischaemia-reperfusion injury or acute rejection, concerns over the effects of the immune response elicited by the vector on the later development of graft vasculopathy were refuted in this study.

CONCLUSIONS

Heterotopically transplanted cardiac allografts from Brown Norway to Lewis rats reveal the presence of CAV at 120 days. *Ex vivo* adenoviral serotype 5 vector perfusion of the donor heart does not affect the later development of allograft vasculopathy.

Acknowledgements

This research was supported by: National Institutes of Health Grant HL66958 P2 And Mayo Clinic and Foundation, Rochester, Minnesota

References

1. Kobashigawa J. What is the optimal prophylaxis for treatment of cardiac allograft vasculopathy? *Curr Control Trials Cardiovasc med* 2000;1 (3):166. [PubMed: 11714434]
2. Valantine HA. The role of viruses in cardiac allograft vasculopathy. *Am J Transplant* 2004;4 (2):169. [PubMed: 14974936]
3. Shirali GS, Ni J, Chinnock RE, et al. Association of viral genome with graft loss in children after cardiac transplantation. *N Engl J Med* 2001;344 (20):1498. [PubMed: 11357152]
4. Ivan D, Frazier OH, Abrams J. Fatal disseminated adenoviral infection in an adult heart transplant patient. *J Heart Lung Transplant* 2004;23 (10):1209. [PubMed: 15477117]
5. Avery RK. Viral triggers of cardiac-allograft dysfunction. *N Engl J Med* 2001;344 (20):1545. [PubMed: 11357160]
6. Hattori T, Shimokawa H, Higashi M, et al. Long-term treatment with a specific Rho-kinase inhibitor suppresses cardiac allograft vasculopathy in mice. *Circ Res* 2004;94 (1):46. [PubMed: 14615290]
7. Sata M, Luo Z, Walsh K. Fas ligand overexpression on allograft endothelium inhibits inflammatory cell infiltration and transplant-associated intimal hyperplasia. *J Immunol* 2001;166 (11):6964. [PubMed: 11359858]
8. Longo CR, Arvelo MB, Patel VI, et al. A20 protects from CD40-CD40 ligand-mediated endothelial cell activation and apoptosis. *Circulation* 2003;108 (9):1113. [PubMed: 12885753]
9. Sun H, Subbotin V, Chen C, et al. Prevention of chronic rejection in mouse aortic allografts by combined treatment with CTLA4-Ig and anti-CD40 ligand monoclonal antibody. *Transplantation* 1997;64 (12):1838. [PubMed: 9422428]
10. Brauner R, Wu L, Laks H, et al. Intracoronary gene transfer of immunosuppressive cytokines to cardiac allografts: method and efficacy of adenovirus-mediated transduction. *J Thorac Cardiovasc Surg* 1997;113 (6):1059. [PubMed: 9202687]
11. Li J, Simeoni E, Fleury S, et al. Gene transfer of soluble interleukin-17 receptor prolongs cardiac allograft survival in a rat model. *Eur J Cardiothorac Surg* 2006;29 (5):779. [PubMed: 16520057]
12. Ye L, Haider H, Jiang S, Ge R, Law PK, Sim EK. In vitro functional assessment of human skeletal myoblasts after transduction with adenoviral bicistronic vector carrying human VEGF165 and angiopoietin-1. *J Heart Lung Transplant* 2005;24 (9):1393. [PubMed: 16143262]
13. Muruve DA. The innate immune response to adenovirus vectors. *Hum Gene Ther* 2004;15 (12):1157. [PubMed: 15684693]
14. Bessis N, GarciaCozar FJ, Boissier MC. Immune responses to gene therapy vectors: influence on vector function and effector mechanisms. *Gene Ther* 2004;11 (Suppl 1):S10. [PubMed: 15454952]
15. Newman KD, Dunn PF, Owens JW, et al. Adenovirus-mediated gene transfer into normal rabbit arteries results in prolonged vascular cell activation, inflammation, and neointimal hyperplasia. *J Clin Invest* 1995;96 (6):2955. [PubMed: 8675667]
16. Zhang HG, Zhou T, Yang P, Edwards CK 3rd, Curiel DT, Mountz JD. Inhibition of tumor necrosis factor alpha decreases inflammation and prolongs adenovirus gene expression in lung and liver. *Hum Gene Ther* 1998;9 (13):1875. [PubMed: 9741426]
17. Pellegrini C, Jeppsson A, Taner CB, et al. Highly efficient ex vivo gene transfer to the transplanted heart by means of hypothermic perfusion with a low dose of adenoviral vector. *J Thorac Cardiovasc Surg* 2000;119 (3):493. [PubMed: 10694608]

18. Ono K, Lindsey ES. Improved technique of heart transplantation in rats. *J Thorac Cardiovasc Surg* 1969;57 (2):225. [PubMed: 4884735]
19. Kawauchi M, Suzuki J, Morishita R, et al. Gene therapy for attenuating cardiac allograft arteriopathy using ex vivo E2F decoy transfection by HVJ-AVE-liposome method in mice and nonhuman primates. *Circ Res* 2000;87 (11):1063. [PubMed: 11090553]
20. Aranda JM Jr, Hill J. Cardiac transplant vasculopathy. *Chest* 2000;118 (6):1792. [PubMed: 11115475]
21. Vassalli G, Gallino A, Weis M, et al. Alloimmunity and nonimmunologic risk factors in cardiac allograft vasculopathy. *Eur Heart J* 2003;24 (13):1180. [PubMed: 12831811]
22. Weis M, von Scheidt W. Cardiac allograft vasculopathy: a review. *Circulation* 1997;96 (6):2069. [PubMed: 9323100]
23. Chen D, Ding Y, Schroppel B, et al. Differential chemokine and chemokine receptor gene induction by ischemia, alloantigen, and gene transfer in cardiac grafts. *Am J Transplant* 2003;3 (10):1216. [PubMed: 14510695]
24. Yap J, Pellegrini C, O'Brien T, Tazelaar HD, McGregor CG. Conditions of vector delivery improve efficiency of adenoviral-mediated gene transfer to the transplanted heart. *Eur J Cardiothorac Surg* 2001;19 (5):702. [PubMed: 11343956]
25. Pellegrini C, O'Brien T, Yap J, Jeppsson A, Tazelaar HD, McGregor CG. Systematic evaluation of distribution of transgene expression after adenovirus-mediated gene transfer to the transplanted heart. *Transpl Int* 1998;11 (5):373. [PubMed: 9787414]
26. Chan SY, Li K, Piccotti JR, et al. Tissue-specific consequences of the anti-adenoviral immune response: implications for cardiac transplants. *Nat Med* 1999;5 (10):1143. [PubMed: 10502817]
27. Johnson P, Carpenter M, Hirsch G, Lee T. Recipient cells form the intimal proliferative lesion in the rat aortic model of allograft arteriosclerosis. *Am J Transplant* 2002;2 (3):207. [PubMed: 12096782]
28. Seipelt IM, Pahl E, Seipelt RG, et al. Neointimal inflammation and adventitial angiogenesis correlate with severity of cardiac allograft vasculopathy in pediatric recipients. *J Heart Lung Transplant* 2005;24 (8):1039. [PubMed: 16102439]
29. Abunasra H, Smolenski RT, Yap J, Jayakumar J, Sheppard M, Yacoub MH. Comparison of two gene transfer models for the attenuation of myocardial ischemia-reperfusion injury following preservation for cardiac transplantation. *Eur J Cardiothorac Surg*. 2006
30. Abunasra HJ, Smolenski RT, Yap J, Sheppard M, O'Brien T, Yacoub MH. Multigene adenoviral therapy for the attenuation of ischemia-reperfusion injury after preservation for cardiac transplantation. *J Thorac Cardiovasc Surg* 2003;125 (5):998. [PubMed: 12771872]
31. Oi K, Davies WR, Tazelaar HD, et al. Ex vivo hypothermic recirculatory adenoviral gene transfer to the transplanted pig heart. *J Gene Med* 2006;8 (7):795. [PubMed: 16652399]
32. Kitagawa-Sakakida S, Fukushima N, Sawa Y, et al. Microarray-based gene expression profiling of retransplanted rat cardiac allografts that develop cardiac allograft vasculopathy. *J Heart Lung Transplant* 2005;24 (12):2068. [PubMed: 16364851]

Figure 1 a – b.

Images of coronary arteries demonstrating the presence of CAV in both Ad-LacZ (top)(**1a**) and UW-perfused (bottom)(**1b**) hearts Morphometric analysis did not show significant differences in occlusion ratios between Ad-Null (0.57 ± 0.22), AdLacZ (0.62 ± 0.19) and UW groups (0.47 ± 0.29) (Data expressed as Mean $\pm$ SD, Analysis of variance)

Figure 2.

Distribution of occlusion ratio for individual rats across the groups. Comparison of the means occlusion ratios between the Ad-Null (0.57 ± 0.22), Ad-LacZ (0.62 ± 0.19) and UW groups (0.47 ± 0.29) groups did not reveal a significant difference ($p=0.511$) (mean $\pm$ SD, Analysis of variance)