CARDIAC AND PULMONARY REPLACEMENT

DIRECT GENE TRANSFER INTO DONOR HEARTS AT THE TIME OF HARVEST

Access to the donor heart at the time of harvest provides a unique opportunity for genetic manipulation of this organ before transplantation. We sought to determine (1) if donor mouse hearts express a foreign gene administered at harvest and, (2) if so, what route of gene delivery is most effective. At harvest, 30 μ g of promoter cytomegalovirus-luciferase deoxyribonucleic plasmid in cationic liposomes was injected directly into the myocardial apex (group I), into the right atrium (group II), or into the coronary arteries (group III). The donor hearts were then transplanted into the abdomen of recipient mice of the same strain. The transplanted hearts were removed in 4 days and luciferase expression was assayed by immunohistochemistry. In group I, luciferase activity was localized to the apex. In group II, where plasmid was delivered into the right atrium, luciferase expression was detected in the right ventricle and sparsely in the coronary perivascular area. In group III, where plasmid was injected into the coronary arteries, the transplanted hearts demonstrated luciferase expression in (1) perivascular areas surrounding coronary arteries and veins, (2) coronary capillaries, and (3) the endocardia of both ventricles. This study suggests that (1) donor mouse hearts can be genetically modified at the time of harvest and (2) intracoronary infusion of plasmid yields the most effective method of delivery. Administration of plasmid in the coronary arteries localizes the expression to the endocardium and the coronary vasculature, both sites of immunologic interactions after heart transplantation. (*J THORAC CARDIOVASC SURG* 1995;109:716-20)

Abbas Ardehali, MD (by invitation), Allistair Fyfe, MD (by invitation), Hillel Laks, MD, Davis C. Drinkwater, Jr., MD (by invitation), Jian-Hua Qiao, MD (by invitation), and Aldons J. Lusa, PhD (by invitation), *Los Angeles, Calif.*

The concept of gene transfer refers to alteration of genetic content of target cells via addition of foreign genes. The development of technology to transfer genetic material into the heart and vasculature of experimental animal models has opened new frontiers in studying the pathogenesis and treatment of cardiovascular diseases. The objective of gene therapy is the efficient delivery of desired genetic material to target cells, resulting in stable

and localized production of a recombinant protein. The feasibility of gene transfer to arterial wall cells has been demonstrated in several animal models with a variety of techniques.¹⁻⁵ However, an optimal system for direct in vivo gene transfer that meets all of these criteria is yet to be developed.

The application of gene transfer to the field of transplantation is uniquely appealing because of access to the donor organ at the time of harvest. It is potentially feasible that a foreign gene may be transferred to the harvested organ, creating a "transgenic" allograft. The application of gene transfer techniques to experimental transplantation has been limited.⁶ We therefore sought to determine (1) if donor mouse hearts express a foreign gene administered at harvest and (2) if so, what route of gene delivery is most effective.

Methods

Construction of expression vector. The firefly luciferase gene was used as the marker gene for this study. The

From the Division of Cardiothoracic Surgery, Department of Surgery, and Division of Cardiology, Department of Medicine, University of California at Los Angeles Medical Center, Los Angeles, Calif.

Read at the Seventy-fourth Annual Meeting of The American Association for Thoracic Surgery, New York, N.Y., April 24-27, 1994.

Address for reprints: Hillel Laks, MD, Division of Cardiothoracic Surgery, UCLA Medical Center, CHS 62-182A, 10833 Le Conte Ave., Los Angeles, CA 90024.

Copyright © 1995 by Mosby-Year Book, Inc.
0022-5223/95 \$3.00 + 0 12/6/61794

plasmid expression vector, pCMV-luciferase, consisted of a full-length firefly *Photinus pyralis* luciferase complementary deoxyribonucleic acid (cDNA) under the control of cytomegalovirus promoter/enhancer.² The vehicle for transfer was cationic liposome. Thirty micrograms of CMV-luciferase DNA plasmid and 50 μ g of cationic liposomes (*N*-[1-(2,3-dioleoyloxy)propyl]-*N,N,N*-trimethylammonium chloride; Lipofectin; Bethesda Research Laboratories, Gaithersburg, Md.) were added to reduced serum medium (Opti-MEM; Gibco Laboratories, Grand Island, N.Y.) to a final volume of 200 μ l. The DNA-liposome solution was prepared approximately 30 minutes before the transfection procedure.

Experimental groups. Both donor and recipient mice were of C57BL/6 strains (Jackson Laboratories, Bar Harbor, Maine). Donor hearts were arrested with cold, dilute (because of high viscosity) University of Wisconsin solution (Du Pont Pharmaceutical, Wilmington, Del.). In group I ($n = 4$), the DNA-liposome solution was directly injected into the myocardial apex on cardiac arrest. The plasmid-liposome solution in group II ($n = 4$) was administered through the inferior vena cava into the right atrium. In group III ($n = 4$), with the distal ascending aorta clamped, the plasmid-liposome solution was directly injected into the ascending aorta over 2 to 3 minutes.

Transplantation. Heterotopic intraabdominal transplantation was performed by means of the technique previously described by Corey, Winn, and Russell.⁷ In brief, donor hearts were arrested with dilute, cold University of Wisconsin solution. The plasmid-liposome solution was administered according to the protocol described earlier. The donor hearts were excised in standard fashion and stored in University of Wisconsin solution until the recipient was prepared. During this time, the transfection medium was in contact with the donor heart. The plasmid-liposome solution was not washed out of the donor organ before transplantation. The heterotopic cardiac transplantation was performed by anastomosing the donor aorta to the side of the recipient infrarenal aorta. An end-to-side anastomosis between the donor pulmonary artery and recipient infrarenal inferior vena cava was performed next. The total ischemic time was 45 minutes to 1 hour, which was also the duration of exposure of donor hearts to plasmid-liposome solution. The function of the transplanted hearts was assessed daily by abdominal palpation. All animals received humane care in compliance with the "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 Institutes of Health (NIH Publication No. 86-23, revised 1985).

Analysis of luciferase expression. The transplanted and recipient hearts were harvested in 4 days when the mice were sacrificed. Multiple 10 μ m thick cross sections of the hearts at the apex and midportion of the ventricle were obtained. Luciferase expression was assayed immunohistochemically with a polyclonal rabbit immunoglobulin G (Cortex Biochem, Inc., San Leandro, Calif.). Immunohistochemical staining was performed in a three-step indirect avidin-biotin immunoperoxidase assay (Vector, ABC Kit,

Table I. Luciferase expression on the donor mouse hearts at the mid-ventricular level

	Group I	Group II	Group III
Right ventricular endocardium	—	+	+
Left ventricular endocardium	—	—	+
Capillaries	—	±	+
Coronary perivascular area	—	±	+

+, Always present; ±, occasionally present; —, absent.

Burlingame, Calif.).⁸ Luciferase expression was assessed by one of us (J.H.Q.) in a blind fashion.

Results

All donor hearts were functioning well at the time of harvest; no acute cellular rejection was apparent on histologic evaluation. Immunohistochemical staining of the donor hearts at the mid-ventricular level for luciferase expression is illustrated in Table I. The mid-ventricular cross sections of the hearts in group I did not reveal any luciferase expression. However, when apical cross sections were examined, luciferase expression could be detected on the apical vasculature and myocytes. In group II, the plasmid-liposome solution was administered through the inferior vena cava into the right atrium; the donor hearts in this group revealed luciferase expression on the right ventricular endocardium and sparse expression around the coronary vasculature. No luciferase expression could be detected on the left ventricular endocardium in group II. In group III, plasmid-liposome solution was injected into the aortic root, perfusing the coronary arteries in an antegrade fashion. Luciferase expression was evident on the left ventricular endocardium, right ventricular endocardium, coronary capillaries, and around the coronary vessels (Figs. 1 and 2).

Although double immunostaining to identify the transfected cell types was not carried out, luciferase expression appeared to be present on endothelial cells, some arterial smooth muscle cells, and some myocytes on review of adjacent sections. The recipient mouse hearts of all groups underwent identical immunohistochemical processing and no luciferase expression could be identified.

Discussion

The findings of this study suggest that donor mouse hearts can be transfected at the time of harvest. Intracoronary infusion of plasmid appeared to be an efficacious route of delivery of reporter

Fig. 1. Mid-ventricular cross section of a donor heart in group III immunostained for luciferase expression. In this group, the plasmid-liposome solution was injected into the aortic root. Luciferase expression was detectable on both ventricular endocardia.

Fig. 2. Luciferase expression on a mid-ventricular cross section of a donor heart in group III. Luciferase activity was detected around coronary vasculature.

plasmid, yielding detectable expression on the endocardia, coronary capillaries, and perivascular area. Expression of the reporter plasmid was localized to the transplanted hearts and did not appear to induce cellular rejection of the isografts.

The utility and importance of gene therapy for several disorders has recently been reviewed.^{9,10} Direct in vivo gene transfer to vascular wall cells represents an ideal system for production of secreted proteins with locally therapeutic effects. Ex-

tension of the principles of experimental gene transfer to heart transplantation has been limited. Wang and associates⁶ studied the expression of reporter genes in a rat cardiac isograft model after direct apical gene injection. They observed stable expression of the reporter gene locally for 2 months. The present study demonstrates that foreign genes can be introduced into the donor hearts at the time of harvest. Among the three tested routes of delivery, the intracoronary administration of expression vector yielded the most widespread and strategically important transfection. Luciferase expression was observed on the endothelial cells and in the perivascular area. The endocardium and perivascular area, the sites of initial host-recipient immunologic interaction, are the ideal sites for introduction of genes intended to alter the immune response. This study further documents that gene transfer to the donor hearts at the time of harvest is safe (no evidence of cellular rejection in transplanted hearts), localized (reporter plasmid expression confined to the donor heart), and feasible.

Application of direct gene transfer techniques to the field of heart transplantation offers several possibilities: (1) studying the role of individual genes separately in immunologic interactions, (2) the development of localized therapeutic measures limited to the donor heart, (3) evaluation of gene regulatory/promoter mechanisms responsible for expression of endogenous proteins such as histocompatibility antigens, and (4) alteration of donor organ "foreignness" by controlling human leukocyte antigen (HLA) expression via antisense oligonucleotide transfer.¹¹ The latter technique allows for transfer of recombinant genes coding for messenger ribonucleic acid (mRNA), which binds to HLA mRNA and effectively blocks HLA expression. Genetic modification of nonhuman hearts to allow xenotransplantation is an established area of investigation.¹² Application of gene transfer technology at the time of donor procurement to the field of xenotransplantation may further the goals of this area of research. Improvements in gene transfer methods to yield efficient delivery techniques, producing biologically effective recombinant protein levels, in specific cellular targets are needed to further the applications of gene therapy in transplantation.

In conclusion, donor mouse hearts can be transfected by a reporter plasmid at the time of harvest. Intracoronary infusion of reporter plasmid yielded detectable expression in perivascular areas and endocardia, both sites of immunologic interaction be-

tween the donor and the host after transplantation. Intracoronary administration of plasmid may allow genetic alteration of donor organs in the near future.

We gratefully acknowledge the technical assistance of Thomas Drake, MD, in photomicrography.

REFERENCES

1. Nabel EG, Plautz G, Nabel GJ. Site-specific gene expression in vivo by direct gene transfer into the arterial wall. *Science* 1990;249:1285-8.
2. Lim CS, Chapman GD, Gammon RS, et al. Direct in vivo gene transfer into the coronary and peripheral vasculature of the intact dog. *Circulation* 1991;83:2007-11.
3. Chapman GD, Lim CS, Gammon RS, et al. Gene transfer into coronary arteries of intact animals with a percutaneous balloon catheter. *Circ Res* 1992;71:27-33.
4. Leclerc G, Gal D, Takeshita S, Nikol S, Weir L, Isner JM. Percutaneous arterial gene transfer in a rabbit model: efficiency in normal and balloon-dilated atherosclerotic arteries. *J Clin Invest* 1992;90:936-44.
5. Guzman R, Lemarchand P, Crystal RG, Epstein SE, Finkel T. Effective and selective adenovirus-mediated gene transfer in vascular neointima. *Circulation* 1993;88:2838-48.
6. Wang J, Jiao S, Wolff JA, Knechtle SJ. Gene transfer and expression in rat cardiac transplants. *Transplantation* 1992;53:703-5.
7. Corey RJ, Winn HJ, Russell PS. Primary vascularized allografts of hearts in mice: the role of H-2D, H-2K, and non-H-2 antigens in rejection. *Transplantation* 1973;16:343-50.
8. Loos CM, Das PK, Oord JJ, Houthoff HJ. Multiple immunoenzyme staining technique: use of fluoresceinated, biotinylated and unlabelled monoclonal antibodies. *J Immunol Methods* 1989;117:45-52.
9. Cournoyer D, Caskey CT. Gene therapy for the immune system. *Annu Rev Immunol* 1993;11:297-329.
10. Nabel EG, Nabel GJ. Direct gene transfer: basic studies and human therapies. *Thromb Haemost* 1993;70:202-3.
11. Helene C, Tolumé JJ. Specific regulation of gene expression by antisense, sense, and antigenic nucleic acids. *Biochem Biophys Acta* 1990;1049:99-125.
12. Markmann JF, Campos L, Bhandoola A, et al. Genetically engineered grafts to study xenoreactivity: a role for indirect antigen presentation in the destruction of major histocompatibility complex antigen deficient xenografts. *Surgery* 1994;116:242-8.

Discussion

Dr. Robert A. Guyton (Atlanta, Ga.). I am confused about the exposure of the plasmid to the donor heart. Was

the plasmid-luciferase combination injected before arrest of the donor heart, that is, while the donor heart was metabolically active, and what was the duration of exposure of the donor heart to the plasmid? Was the donor heart at 4° C while the plasmid was exposed to the donor heart?

Dr. Ardehali. The donor hearts were arrested with

University of Wisconsin solution; the plasmid-liposome solution was then administered according to the described protocol. The allografts were not flushed thereafter. The hearts were then preserved in cold University of Wisconsin solution for 45 minutes to 1 hour until the recipients were prepared. Therefore, transfection occurred during the cold ischemic time (45 minutes to 1 hour).

Bound volumes available to subscribers

Bound volumes of THE JOURNAL OF THORACIC AND CARDIOVASCULAR SURGERY are available to subscribers (only) for the 1995 issues from the Publisher, at a cost of \$91.00 for domestic, \$118.77 for Canadian, and \$111.00 for international subscribers for Vol. 109 (January-June) and Vol. 110 (July-December). Shipping charges are included. Each bound volume contains a subject and author index and all advertising is removed. Copies are shipped within 60 days after publication of the last issue of the volume. The binding is durable buckram with the JOURNAL name, volume number, and year stamped in gold on the spine. *Payment must accompany all orders.* Contact Mosby-Year Book, Inc., Subscription Services, 11830 Westline Industrial Drive, St. Louis, Missouri 63146-3318, USA; phone 1 (800) 453-4351 or (314) 453-4351.

Subscriptions must be in force to qualify. Bound volumes are not available in place of a regular JOURNAL subscription.