Transpl Int (1998) 11: 373–377

ORIGINAL ARTICLE

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Systematic evaluation of distribution of transgene expression after adenovirus-mediated gene transfer to the transplanted heart

Received: 9 December 1997
Received after revision: 24 March 1998
Accepted: 15 April 1998

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Abstract In the transplantation setting, the study and potential treatment of acute and chronic rejection by means of gene therapy will require widespread transgene expression in the donor organ. The distribution of transgene expression after adenovirus-mediated gene transfer via the coronary vasculature in a model of abdominal heterotopic heart transplantation in syngeneic rats ($n = 6$) was evaluated at 1 week. Reporter gene expression was evenly distributed in the base, the midventricle, and the apex of the transplanted hearts. This study demonstrates that intracoronary administration of the adenoviral vector to the donor heart results in widespread transgene expression.

Key words Adenovirus, heart, transplantation · Gene therapy, heart transplantation · Heart transplantation, gene therapy · Gene transfer, heart transplantation

Introduction

In the setting of heart transplantation, the main limiting factor to long-term survival is accelerated graft atherosclerosis [13]. The possibility of genetic manipulation of the donor heart by means of gene therapy provides the potential for evaluating the cellular and molecular mechanisms involved in this pathological process and may have therapeutic potential. Moreover, gene transfer may afford the means for organ-specific immunosuppression by altering the antigenicity of the graft, thus minimizing the need for systemic administration of immunosuppressive agents [6]. To achieve these goals, a gene transfer method that ensures even and widespread transgene expression into the donor organ is necessary. This study was designed to examine the distribution of transgene expression in the transplanted heart after intracoronary administration of an adenoviral vector ex vivo.

Materials and methods

Adenovirus vector

A replication-defective, E1 a-deleted serotype 5 adenoviral vector encoding for *Escherichia coli* β-galactosidase, under the control of the CMV promoter, was used in this study (AdCMVLacZ, a kind gift of James Wilson, Institute for Gene Therapy, University of Pennsylvania). The recombinant virus was propagated in transformed human embryonic kidney carcinoma cells ("293 cells"), isolated, purified by ultracentrifugation through two cesium chloride gradients, dialyzed against 140 mM NaCl, 0.5 M Hepes, and

1 M $MgCl_2$ for 3.5 h at 4 °, and stored at – 70 °C in 10 % glycerol until use. Viral titers were determined by plaque assay and expressed as plaque-forming unit per ml (pfu/ml).

Operation and gene transfer

Inbred male Lewis rats weighing 250–300 g (n = 12) were used as donors and recipients for syngeneic transplants. 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).

Heterotopic abdominal heart transplantation using standard microsurgical techniques was performed [12]. After anesthesia, the donor rat was intubated and ventilated (Harvard Rodent Ventilator). A median sternotomy was performed to expose the heart. After injection of 200 units of aqueous heparin into the inferior vena cava, the innominate artery was cannulated with a 24-gauge cannula and the venae cavae and pulmonary veins were ligated en bloc with 4/0 silk. The aorta was tied distal to the cannula and the heart was arrested with an infusion of cold cardioplegic solution (Plegisol, Abbott Laboratories, Abbott Park, Ill.) into the aortic root via the indwelling cannula. After harvesting, the heart was stored in the same cardioplegic solution at 4 °C. A volume of 350 µl of viral solution at a titer of 1×10^9 pfu/ml was infused over 5 s into the coronary arteries via the aortic root. The pulmonary artery was clamped during infusion and the virus was not flushed out at the end of 60 min of cold storage. All donor hearts were heterotopically transplanted into the recipients by end-to-side anastomoses of the aorta and the pulmonary artery to the abdominal aorta and inferior vena cava, respectively, using 10/0 monofilament sutures. All rats received analgesia post-operatively and recovered with oxygen in a warm environment. Function of the graft was checked daily by palpation of the beating, transplanted heart.

Assessment of transgene expression

After 1 week, the animals were sacrified with an intraperitoneal injection of pentobarbital sodium (70 mg/kg) and the transplanted hearts were removed and flushed with saline. A basal, a midventricular, and an apical section (about 5 mm each) were separately embedded in OCT compound (Miles, Elkart, Ind.) and snap-frozen in a liquid nitrogen-cooled isopentane bath. Fifteen 5-µm-thick cryostat sections were then cut at 100-µm intervals for each portion. Specimens were fixed in 1.25 % glutaraldehyde for 15 min at 4 °C and then rinsed twice with phosphate-buffered saline (PBS; Gibco BRL, Gaithersburg, Md.). Sections were stained in a solution of 500 µg/ml 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal; Boehringer, Mannheim, Indianapolis, Ind.) for 4 h at 37 °C. The specimens were rinsed in PBS and counterstained with eosin. Blue-stained cells indicated the presence of β-galactosidase expression. For quantitative analysis, the total number of positive staining cells was counted manually for each section under magnification (× 200) and normalized to the area. The normalized values for basal, mid-ventricular, and apical portions were calculated. The area of each section was obtained using an Axiophot Photomicroscope (Carl Zeiss, Oberkochen, Germany) equipped with a 2.5 × objective lens. Brightfields images were digitized to 256 gray levels on an IBAS Image Analysis System (Kontron Elektronik, Munich, Germany) using a black and white Newvicon Video Camera (Hamamatsu, Tokyo, Japan). Each image was analyzed using a macro-computer program written with software supplied by the IBAS system.

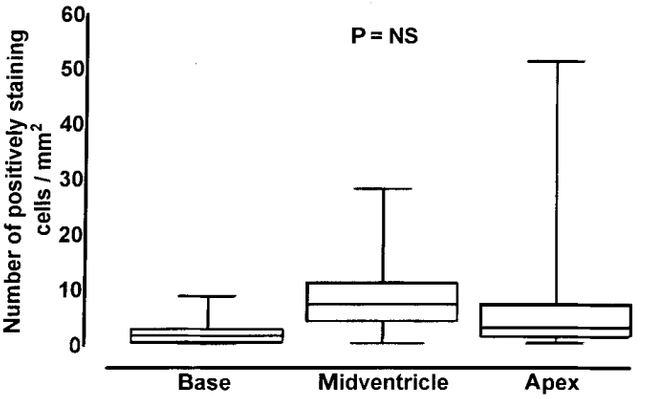

Fig. 1 Distribution of transgene expression in the basal, midventricular, and apical sections of the transplanted heart. The *boxes* represent the 25th, median, and 75th percentiles whereas *whiskers* denote the range of the number of cells positively staining for β-galactosidase/mm² of tissue. No significant difference was observed (n = 6)

Statistical analysis

Results are expressed as median and range. As data did not follow a Gaussian distribution and variances were unequal, a nonparametric test of analysis of variance (Friedman test) was performed to evaluate differences between the three areas examined. A P value of less than 0.05 was considered significant.

Results

There was no operative mortality. All transplanted hearts were beating at the time of harvest. Transgene expression was detected in all portions of the heart. The number of positive cells per mm² of tissue was: base 1.807 (range 0.205–8.936); mid-ventricle 7.559 (range 0.482–28.4); apex 3.291 (range 0.613–51.7; Fig. 1). No significant differences were found between the three groups. All cells types (myocytes, endothelial cells, and interstitial fibroblasts) stained positively for β-galactosidase. Myocytes were the most frequently transduced cells (Fig. 2), whereas only rare endothelial cells expressed the transgene protein (Fig. 3). Staining appeared more accentuated in the subepicardial region and around zones of organizing ischemia.

No acute inflammation was associated with foci of transgene expression.

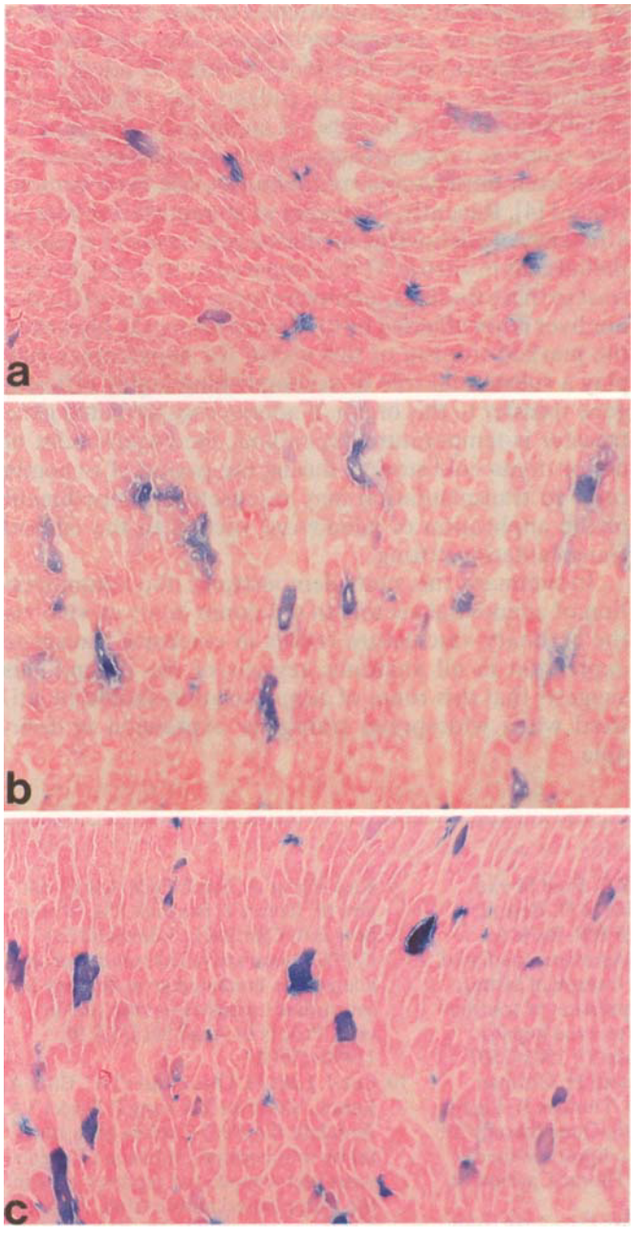

Fig. 2 a–c Demonstration of transgene expression by histochemical staining for β-galactosidase in the: **a** basal, **b** midventricular, and **c** apical portions of the heart. The positively staining cells are blue (all magnification × 50)

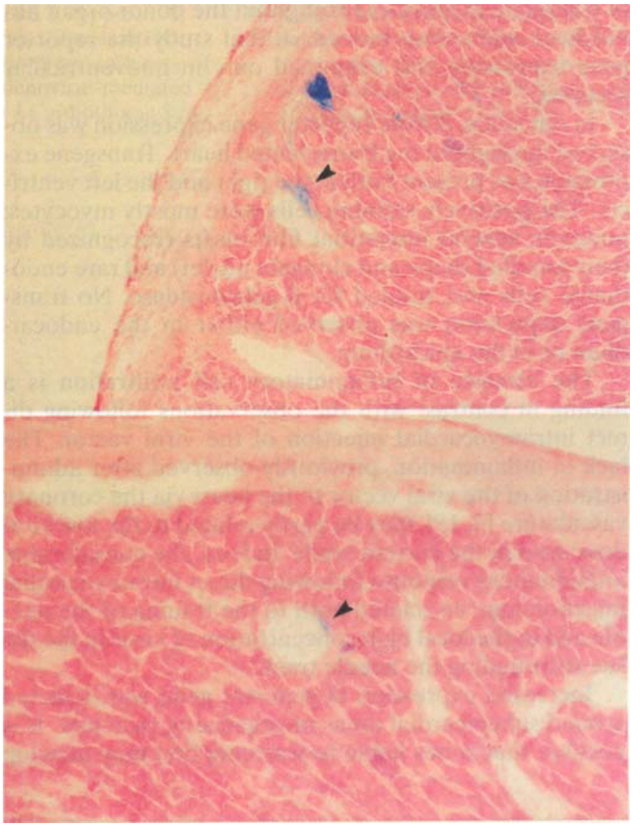

Fig. 3 Demonstration of transgene expression by histochemical staining for β-galactosidase in endothelial cells. The positively staining cells are blue *(arrows)*. Transduced endothelial cells were rare compared to cardiomyocytes

Discussion

The present study demonstrates that intracoronary administration of adenoviral vectors to the donor heart results in widely distributed transgene expression.

Two different routes of vector delivery have been used to achieve gene transfer to the transplanted heart: (a) direct injection into the myocardium [1], and (b) infusion via the coronary vasculature [10]. The former method is able to accomplish efficient gene transfer, but transgene expression is limited to an area within 1–2 mm of the needle track and is associated with a marked inflammatory cell infiltrate [7–9]. However, intramyocardial injection of the vector may be effective in treating localized pathological processes affecting the myocardium. Recently, recombinant adenoviruses encoding for the vascular endothelial growth factor have been used to induce collateral blood vessel development in the ischemic myocardium, thus obtaining the restoration of blood flow and enhancing myocardial function [11]. Localized gene therapy with angiogenic factors may, therefore, be a therapeutic option for myocardial ischemia. To approach more generalized processes such as acute and chronic rejection of cardiac allografts, however, widespread transgene expression will be required. While efficient gene transfer to the donor heart via intracoronary infusion of an adenoviral vector has previously been demonstrated [10], the distribution

of transgene expression throughout the donor organ has not been addressed. Indeed, in that study the reporter gene expression was examined only in midventricular sections.

In our study, diffuse reporter gene expression was observed throughout the transplanted heart. Transgene expression was present both in the right and the left ventricle. The positively staining cells were mostly myocytes; however, several interstitial fibroblasts (recognized by their spindled shape and elongate nuclei) and rare endothelial cells also stained for β-galactosidase. No transgene expression was observed either in the endocardium or in the epicardium.

The absence of inflammatory cell infiltration is a finding in contrast with the observations following direct intramyocardial injection of the viral vector. The lack of inflammation, previously observed after administration of the viral vector to the heart via the coronary vasculature [2, 15], may be partly related to the low viral dose used in the current study. Indeed, the considerable inflammatory response following direct intramyocardial injection may be related both to the trauma of the needle and to the local high concentration of virus in the tissue surrounding the needle track.

Increased expression of reporter gene was detected around subepicardial areas of organizing ischemia. It is possible that zones liable to warming first may develop ischemia and that this accentuated transgene expression in peri-ischemic areas may suggest the relevance of warm ischemia, and thus temperature, to adenoviral-mediated gene transfer [14]. Indeed, less efficient viral penetration of cells, because of impairment of temperature-sensitive processes such as endocytosis and viral release from intracytoplasmic vacuoles, has been demonstrated [4]. Recently, Chapelier et al. reported enhanced efficiency of adenovirus-mediated gene transfer to endothelial cells in vitro at 37 °C compared to 10 °C [3]. In contrast to these findings, adenoviral gene transfer to the donor liver is not affected by low temperature [5]. However, the marked tropism of this vector for hepatocytes may play a substantial role in accomplishing highly efficient gene transfer to this organ. If adenovirus-mediated gene transfer is temperature-dependent, the requirement of donor organ cold storage during the period of ischemia prior to transplantation may represent an impediment to the application of adenoviral vectors to the clinical transplantation setting.

In summary, this study demonstrates that administration of adenoviral vectors to the donor heart ex vivo via the coronary vasculature results in extensive transgene expression in all anatomic regions of the organ. This suggests that this route of administration should be utilized when widespread transgene expression is desirable.

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