Graft Function After Heterotopic Rat Heart Transplant With an Isolated Reperfused Working Heart: A Methodic Consideration

Dominik Wiedemann,1, 3 Florian Boesch,2 Stefan Schneeberger,2 Alfred Kocher,3 Günther Laufer,3 Severin Semsroth1

Abstract

Objectives: Assessment of graft function in experimental cardiac transplant has been underused by insufficient methods (echocardiography, magnetic resonance imaging). The isolated reperfused working-heart model is an excellent tool for hemodynamic evaluation of rodent hearts. So far, it has never been used in a cardiac transplant setting. Our study tries to combine the in vivo technique of a rat heart transplant model with the ex vivo method of an isolated working heart.

Materials and Methods: Heterotopic heart transplants have been performed in rats with a nonsuture cuff technique. After 8 hours of cold ischemia and 24 hours of reperfusion, grafts were mounted on the working heart. To assess graft function, cardiac output was measured with increasing levels of afterload pressure. Nontransplanted hearts were mounted directly to the working heart apparatus to serve as a control group. Each heart was assessed subjectively by the Stanford score before being mounted on the working-heart apparatus.

Results: The working-heart assessment detected significantly impaired graft function in the transplant group compared with control hearts. In contrast, functional assessment with the score-system could not detect any difference between transplanted and native hearts.

Conclusions: The isolated working-heart model is an excellent tool for assessing graft function after experimental heart transplant in rodents.

Key words: Working heart, Ischemia/reperfusion, Heart transplant

Introduction

In the field of experimental transplant research, different rodent heart transplant models1–3 have been used to evaluate ischemia/reperfusion (I/R) and rejection. Although such investigations brought interesting morphologic and biochemical results,4–7 functional evaluation has always been rather insufficient and nonobjective. Score systems, like the Stanford Score described by Blanchard and Pollad (Figure 1), are subjective and not very sensitive. Echocardiography and magnetic resonance tomography are used to assess cardiac function in small animals, but not in transplant experiments. This is because these methods depend on atrial and ventricular filling for hemodynamic assessment (ejection fraction, fractional shortening, end diastolic volume). The criterion standard for the assessing cardiac function in small animals (rabbits, rats, and mice) is the isolated working heart, as described by Neely.8 The working heart apparatus has been used to assess cardiac function in different heart failure models, for example, left anterior descending artery ligation.9

So far, the working heart model has not been described to evaluate graft function after cardiac transplant. Reasons for this might be the high logistic effort, the difficulties of both techniques (the transplant and the working heart preparation), and its combination. Our goal was to combine the in vivo
technique of a heterotopic heart transplant model and the ex vivo technique of the isolated reperfused working heart to establish a new possibility to evaluate graft function after heart transplant.

Materials and Methods

Cardiac transplant model
Hearts of male inbred Lewis rats (Harlan Winkelmann, Borchen, Germany) weighing 250 to 350 g were used for transplant experiments. Animals were held under standard conditions with unlimited access to water and standard laboratory feed according to local guidelines and the Austrian Animal Care Law. All experiments were performed with the approval of the National Animal Welfare Committee. Anesthesia for transplant procedures was done with intramuscular injection of 100 mg/kg ketamine (Ketavet, Pharmacia & Upjohn, Erlangen, Germany) and 5 mg/kg xylazine (Xylasol, Dr. E. Graeub AG, Bern, Switzerland). Hearts were transplanted heterotopically into the neck of the recipients (n=8). The technique we used was a nonsuture cuff technique described by Heron and associates,3 modified, and ameliorated in the Daniel Swarovski Research Laboratory, Medical University, Innsbruck, Austria. Basically, we connected the aorta of the graft with the common carotid artery of the recipient and the graft’s pulmonary trunk with the recipient’s external jugular vein over polyethylene cuffs. This model is a “reperfusion only” model without filling of the atria or the ventricles. Native rat hearts that were not transplanted served as control group (n=8).

Working heart preparation
Experimental groups are shown in Figure 2. Hearts were flushed with chilled (0°C to 1°C) Custodial preservation solution, stored for 8 hours in the same solution, and transplanted into syngenic recipients. After 24 hours of in vivo reperfusion, graft function was assessed with the Stanford Score, by 2 independent investigators (D.W. and F.B.) (Table 1). Under terminal anesthesia, the animals were anticoagulated with heparin (500 IV IU). Grafts were recovered and immediately placed into ice-cold saline. Afterwards, we cannulated the aorta for a Langendorff retrograde perfusion (80 mm Hg perfusion pressure) with warm (37°), oxygenated Krebs-Henseleit-Buffer. The time required to mount the heart for Langendorff perfusion was standardized to 7 minutes. Krebs-Henseleit-Buffer (pH 7.4) consisted of (in millimoles per liter): NaCl 118; KCl 4.7; CaCl2 2.5; MgSO4 1.2; KH2PO4 1.2; NaEDTA 0.5; NaHCO3 25; and glucose 11.1. Additional 2.5 IU/L insulin and 2 g/L bovine albumin were added to prevent edema.

After 15 minutes’ establishment of coronary perfusion over the aortic cannula in the Langendorff mode, the pulmonary veins were reopened with a cut in the confluens of the left and right pulmonary veins. A venous cannula was inserted in the left atrium. This cannula was connected to a preload reservoir of the perfusion apparatus. After 10 minutes of stabilization with retrograde perfusion, hearts were exposed to the working heart mode. The left atrium was perfused with a standardized preload pressure of 8 mm Hg, while the left ventricle ejected against a standardized afterload pressure of 80 mm Hg. A pacing electrode was attached to the right atrium and hearts were paced at 230 beats/minute. A tip-catheter (Millar Instruments, Inc., Houston, Texas, USA) was inserted into the left ventricle for measurement of the left ventricular pressure.

Evaluation of graft function – pump function curves (pressure-volume)
After a stabilization period of 5 minutes at standard preload and afterload pressure, the afterload pressure was reduced from 80 mm Hg to 10 mm Hg and the ventricular pressure and the cardiac output were measured. Every 5 seconds, the afterload pressure was raised by steps of 10 mm Hg, and the cardiac output and the ventricular pressure were measured at every time point, until the cardiac output reached 0.

Results
Comparing the transplanted hearts after 8 hours’ cold ischemia and 24 hours’ in vivo reperfusion with native rat hearts, the Stanford Score showed nearly
normal or only slightly impaired cardiac function in the transplant group (4 [3 to 4] vs 4 [4 to 4]). However, this difference did not reach statistical significance ($P = NS$) (Figure 1).

In contrast to these results, the working heart investigation could detect clear signs of myocardial insufficiency in the transplant group (Figure 2). The pump function curve of the transplanted hearts shows the typical left and downward shift that is a remarkable sign of myocardial damage. The increasing afterload pressure results in diminishing levels of cardiac output. In contrast, the pump function curve of the native rat hearts shows no signs of myocardial insufficiency.

**Discussion and Conclusions**

These experiments show a new possibility in assessing cardiac function after organ transplant in rats. Especially, the sensitivity of the isolated working heart preparation for hemodynamic evaluation is quite beneficial for assessing ischemia reperfusion injury, as well as in rejection experiments. This could be clearly demonstrated by discrimination of hemodynamic functions in hearts, although the Stanford score was almost normal. The challenge of this technique is mainly logistical; you need both: a heart transplant model and the Langendorff/Working Heart model.

A major technical difficulty is cannulating the left atrium. During the transplant procedure, the pulmonary veins are ligated, but during cannulation, reopening is inevitable. Therefore, we opened the confluens of the pulmonary veins to facilitate cannulation. Other difficulties were postoperative adhesions that had to be liberated carefully.

The transplant model we used is a “reperfusion only” model, so there is no filling of the atria and ventricles during the 24 hours of in vivo reperfusion. This could have adverse effects on myocardial function additional to ischemia reperfusion injury. Other options for evaluating cardiac function are echocardiography and magnet resonance imaging (MRI). These techniques have been used in rodent models. For example, after myocardial infarction in the rat, the functional deficit of the grafts is evaluable by means of ultrasound and MRI. But it remains questionable if echocardiography or MRI can detect graft function in transplant models. Most of the models used for transplant experiments are heterotopic transplant models, like the one we used for our experiments. All of these models are reperfusion models only, so that it seems impossible to evaluate hemodynamic parameters, for example, ejection fraction.

In a previous publication, we already evaluated the protective effect of a fibrin derived peptide (B-beta 15-42) on ischemia-reperfusion injury after heart transplant in rats with the isolated reperfused working-heart. In this study, we could detect significant functional differences between treated and untreated grafts.\(^{10}\)

In summary, our experimental feasibility study demonstrates a combination of an in vivo heart transplant model with the isolated reperfused working-heart preparation. These presented results could complete experimental approaches and results in heart transplant research.

**References**


