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Non-volume-loaded heart provides a more relevant heterotopic transplantation model

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ABSTRACT

Background: We aimed to compare two techniques of heterotopic heart transplantation in rats. Non-volume-loaded (NL) and volume-loaded (VL) models were tested for their physiologic and immunologic properties to assess their suitability for transplant studies.

Methods: Syngeneic heterotopic heart transplants were performed according to the techniques previously described by Ono (NL) and Yokoyama (VL). Grafts were followed over 90 days with sequential echocardiography. Ex-vivo Langendorff perfusion was used to gain functional data. Allogeneic heart transplants were done to determine whether chronic allograft vasculopathy (CAV) develops at a different pace in both transplant models.

Results: The ischemic time during surgery was significantly longer using the VL model ($p<0.001$). The LV diameter of NL hearts decreased over time while that of the VL model significantly increased ($p=0.004$ on POD 90). Mean LV developed pressure and (dP/dt)max were significantly higher with the NL model (61.1 ± 8.5 mmHg and 4261.7 ± 419.6 mmHg/s) than with VL hearts (19.9 ± 16.5 mmHg; $p=0.011$ and 924.8 ± 605.6 mmHg/s; $p<0.001$). The mean weight of NL hearts (0.45 ± 0.03 g) was significantly less than that of VL hearts (1.21 ± 0.16 g, $p<0.001$). Histology of syngeneic NL grafts showed healthy, but partly atrophic myocardium, whereas the LV myocardium of VL hearts showed dilation and scarring typical for chronic ischemic injury. Heart allografts similarly developed CAV with luminal narrowing of $37.2\pm16.6\%$ (NL) and $34.4\pm21.4\%$ (VL), respectively by POD 90 ($p=0.807$).

Conclusions: Since the coronary arteries in the VL model get perfused with partly deoxygenated blood, the myocardium suffers from chronic ischemic injury. We recommend using the NL model in preclinical transplant studies.

1. Introduction

Survival after heart transplantation steadily improves with successive eras and current 1-year and 10-year survival rates are approximately 85% and 50%, respectively. However, the major improvements have been limited to the first 6 to 12 months postoperatively without affecting the annual mortality thereafter. The survival continues to decrease at a very linear rate of approximately 3.4% per year after the first 12 months [1]. Chronic allograft vasculopathy (CAV) remains the major obstacle to long-term survival in heart transplantation and occurs with a cumulative prevalence of 32.1% and 44.3% at 5 and 8 years, respectively [2]. Survival for patients with documented CAV has been shown to be significantly worse compared to patients without CAV [1].

To improve long-term clinical outcomes, treatment strategies must be effective in reducing CAV. Therefore, two different heterotopic heart transplant models in rats have been established. The most commonly used model was first described by Abott and subsequently modified by Ono and Lindsey [3,4] and was used by 87% of published studies since 2000. The remaining 13% of experiments used the model introduced by Yokoyama in 1995 [5]. The central distinction between the two models is the blood volume loading of the left ventricle. In the Ono model, the LV is non-volume-loaded (NL), whereas Yokoyama's modification results in a volume-loaded (VL) heart. The question of adequacy has been raised [6] and our study aims to compare the immunology, physiology, and morphology of both models.

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2. Materials and methods

2.1. Animals and heterotopic heart transplants

Male PVG and ACI rats weighing 250 g were purchased from Harlan (Indianapolis, IN, USA) and were housed under conventional conditions at the animal care facilities of the Department of Cardiothoracic Surgery, Stanford University Medical Center, California. The rats were fed standard rat chow and water ad libitum. 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 published by the National Institutes of Health (National Institutes of Health publication 85-23, revised 1985). Heterotopic heart transplants were performed by one experienced microsurgeon as shown in Fig. 1. In the NL model, the donor ascending aorta and main pulmonary aorta were anastomosed to the infrarenal abdominal aorta and vena cava inferior (IVC) of the recipient, respectively. In the VL model, an ASD was created and the leaflets of the tricuspid valve were resected to produce an insufficiency. The pulmonary artery was ligated. The donor aorta was anastomosed to the recipient's abdominal aorta and the donor right atrium was anastomosed to the IVC.

2.2. Study design

Six syngeneic (ACI to ACI) and 6 allogeneic (PVG to ACI) transplants were performed with the NL and VL models each. Recipients of allogeneic hearts received cyclosporine A (10 mg/kg) per oral gavage for 7 days. All studies on graft physiology (echocardiography and Langendorff perfusion) were done on syngeneic grafts only to avoid bias by immunologic graft injury. Allografts were used to compare the development of cardiac allograft vasculopathy (CAV) between the models. All grafts were harvested after 90 days.

2.3. Graft physiology

2.3.1. Echocardiography

Transplanted hearts were imaged serially at postoperative days (POD) 15, 30, 45, 60, and 90. On each day, animals were anesthetized with isofluorane (3.5%), shaved, and positioned supine on the echocardiography platform with their head in a nose cone for continuous anesthesia with isofluorane (2.5–3.5%). Ultrasound images were obtained using a GE Healthcare Vivid 7 Ultrasound as previously described [8,9].

2.3.2. Langendorff perfusion

Transplanted hearts were explanted and analyzed using a Langendorff apparatus as previously described [7]. Briefly, rats were heparinized (2000 U/kg, IP) and anesthetized with sodium pentobarbital (100 mg/kg, IP). Transplanted hearts were isolated, affixed to the Langendorff apparatus, and perfused with oxygenated Krebs solution containing (in mM) 120 NaCl, 5.8 KCl, 25 $NaHCO_3$, 1.2 NaH_2PO_4, 1.2 $MgCl_2$, 1.8 $CaCl_2$, and 10 dextrose, pH 7.4 at 37 °C. A latex balloon was inserted into the LV and inflated until 6–8 mm Hg of LV end diastolic pressure (LVEDP) was achieved. The pressure tracing

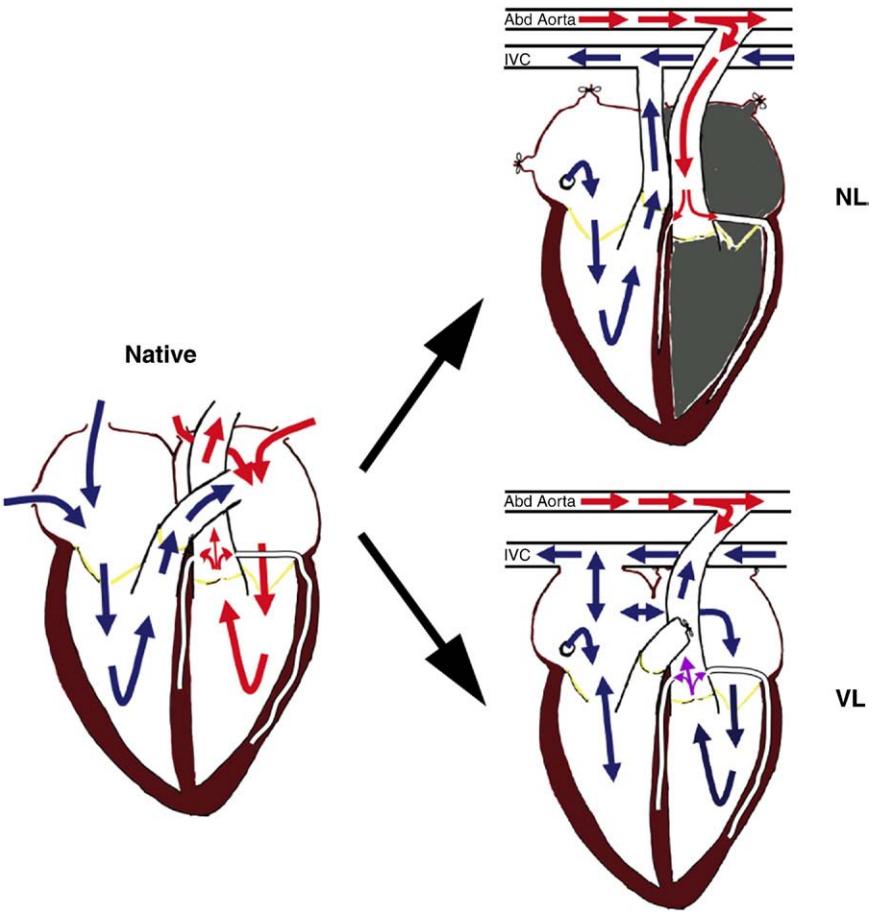

Fig. 1. Schematic overview of the two different heart transplant models. The red arrows indicate the direction of oxygenated blood flow, while blue and purple arrows indicate deoxygenated and mixed blood flows, respectively.