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A Heterotopic Cardiac Transplantation Model for Evaluation of Rejection Using Transvenous Endomyocardial Biopsy

Taijiro SUEDA[1], Yuichiro MATSUURA[1], Takeshi MATSUSHIMA[1], Shogo MUKAI[1] and Hiroki KAJIHARA[2]

1) The First Department of Surgery, Hiroshima University School of Medicine, 1-2-3, Kasumi, Minami-ku, Hiroshima 734, Japan
2) Organizing Office for College of Medical Technology Hiroshima University, Hiroshima 734, Japan

ABSTRACT

A model of heterotopic cardiac transplantation for diagnosis of rejection is described. Heterotopic cardiac transplantations were performed in the thorax using the left innominate artery as an arterial supply with venous return into the superior vena cava. Six pairs of mongrel dogs underwent cardiac transplantation using this technique. Two dogs died postoperatively on the 2nd and 3rd day due to respiratory failure. Another four donor hearts arrested their beats in 6 to 8 postoperative days (mean 6.3 days) resulting from acute cardiac rejection. Serial echocardiographic recordings were found to be a reliable measure of acute cardiac rejection, since the left ventricular wall thickness of the donor heart increased until the donor heart stopped by rejection. Endomyocardial biopsy was easy to perform by passage of flexible cardiac biotome into the right ventricle of the donor and the recipient heart through the right internal jugular vein. Pathological findings revealed that early changes of the donor heart were interstitial edema caused by myocardial ischemia. This was followed by lymphocyte infiltration around peripheral coronary arteries and acute rejection resulting in myocyte necrosis.

Key words: *Heterotopic cardiac transplantation, Acute cardiac rejection, Endomyocardial biopsy*

Although orthotopic cardiac transplantation is ideal for evaluation of mechanism of acute rejection, long-term survial in animal experiments often proves difficult. In particular, lung edema after extracorporeal circulation is a major cause of mortality in the dogs. Heterotopic cardiac transplantation in animals has several advantages, such as the non-use of extracorporeal circulation, low experimental cost, and ease of hemostasis owing to the non-use of heparin. In a previous study, the authors described a heterotopic cardiac transplantation model which maintained pulmonary and systemic circulations by the only donor heart[7]. In order to evaluate acute rejection process, using the technique of endomyocardial biopsy in particular, another simple model of heterotopic cardiac transplantation was devised. This paper describes a new experimental model of heterotopic cardiac transplantation for the study of rejection and serial results of acute rejection process examined by echocardiography and endomyocardial biopsy.

MATERIALS AND METHODS

Six pairs of mongrel dogs (BW 8-15 Kg) were prepared. Smaller dogs were selected as donors and the donor hearts were harvested after cardioplegic arrest. Atrial septotomy of the donor heart was performed before implantation and the donor heart was immersed in iced saline. The recipient dogs underwent thoracotomy in the fourth intercostal space; the superior vena cava and the left innominate artery were dissected. The pulmonary artery of the donor heart was anastomosed to the superior vena cava of the recipient, then the ascending aorta of the donor heart was sutured to the left innominate artery of the recipient. After coronary blood flow was reperfused to the donor heart and vigorous resucitation of the donor heart was obtained, the chest was closed with the thoracic drainage. The sheath catheter (5 F. size) was inserted into the right external juguler vein as the route of endomyocardial biopsy (Fig. 1). Serial echocardiographic evaluation was performed as the non-invasive method of diagnosis of rejection until the donor heart stopped by acute rejection. In particular, the posterior wall thickness of the left ventricle was estimated daily. Endomyocardial specimens of the right ventricle of the donor heart were picked up with flexible biopsy forceps (Cordis Corp.) for the microscopic examination. The specimens were stained using hematoxylin and eosin. Arrest of the donor heart was recognized with external palpation and electrocardiogram.

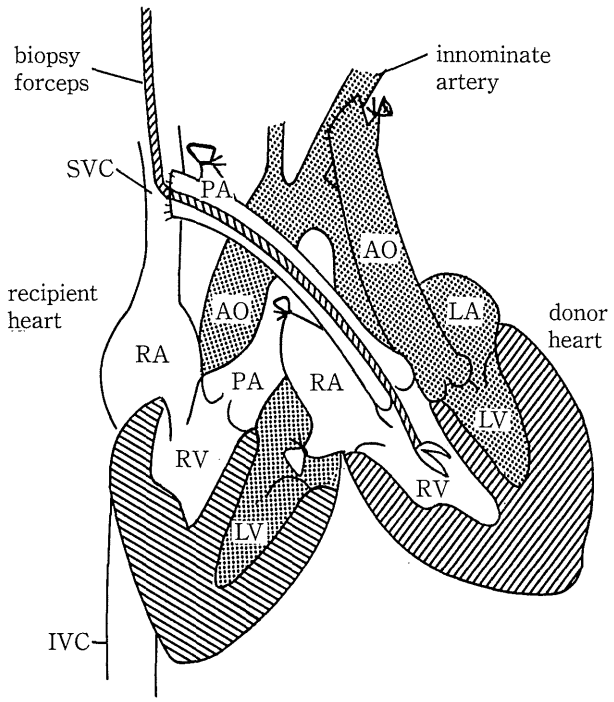

Fig. 1. A heterotopic cardiac transplantation model for endomyocardial biopsy.

The ascending aorta and the pulmonary artery of the donar heart were anastomosed to the innominate and the superior vena cava of the recipient, respectively. 5 F. biotome was able to be introduced into the right ventricle of the donor heart through the superior vena cava.

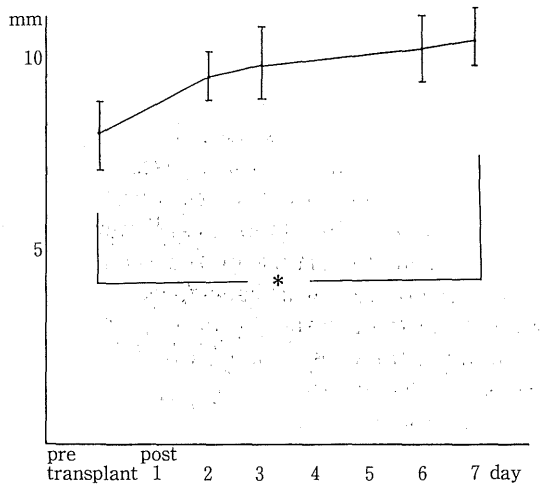

Fig. 2. Increase of the wall thickness, measured from the posterior wall end-diastolic images.

Values indicate the mean ± SD of the wall thichness in 4 donor hearts.
(— * — means significant value. $p<0.005$)

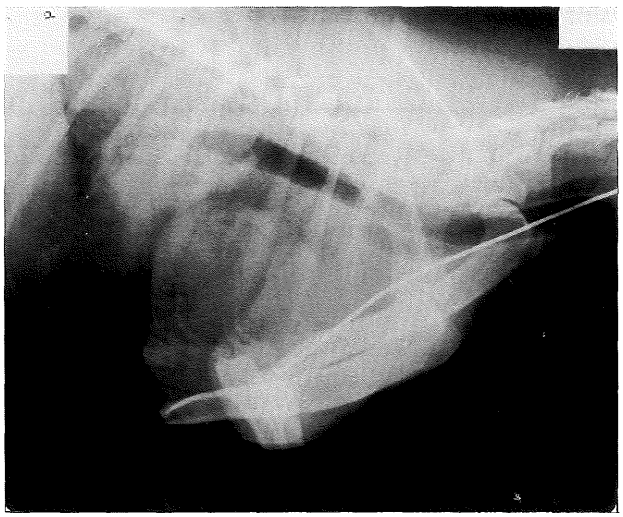

Fig. 3-a

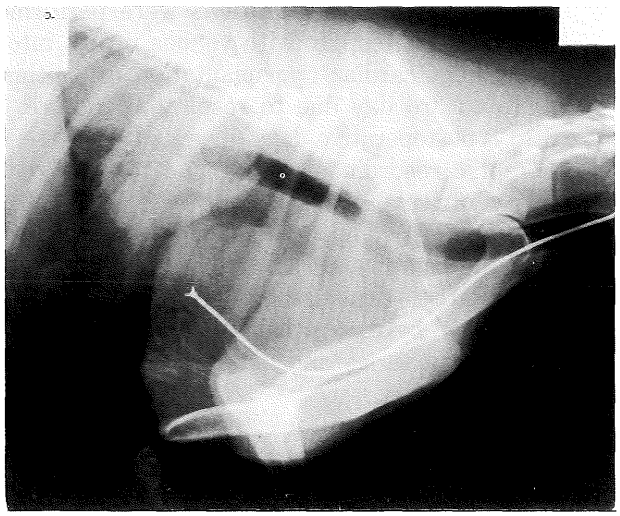

Fig. 3-b

Fig. 3. Endomyocardial biopsies of the donor and the recipient hearts under fluoroscopy.
5 F. flexible biotome was inserted into the right ventricle of the donor heart through the superior vena cava and the pulmonary artery (Fig. 3-a). In addition, the biopsy specimens were taken from the right ventricle of the recipient as controls (Fig. 3-b).

RESULTS

All six donor hearts recovered their beats spontaneously after declamping. Mean ischemic time was 121 min in cold preservation and 38 min in anastomosis. Two donor hearts discontinued their own beats within 3 days after transplantation because of myocardial damage during cardioplegic arrest. Another four hearts continued cardiac beating until cessation due to acute cardiac rejection (mean 6.3 days after transplantation).

Echocardiographic images of the transplanted hearts demonstrated an increase in the brightness of the left ventricular wall through posttransplant days until the hearts were totally rejected. Although this increase was not calculated numerically with two-dimensional echocardiographic images, the findings were consistent in all transplanted hearts. Wall thickness, measured from the posterior wall end-diastolic images, progressively increased. Although the mean thickness was 8.1+0.3mm on the day of transplantation, the

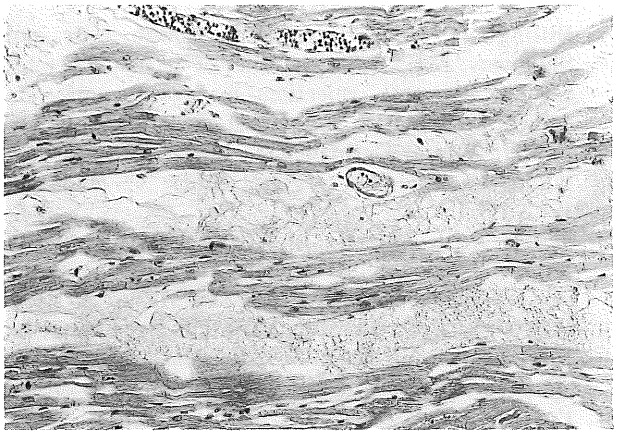

Fig. 4. Biopsy specimen obtained on day 2 after transplantation. (Hematoxylin and eosin stain ×160)
Interstitial edema was prominent and minimal lymphocytic infiltrates were observed in interstitial spaces.

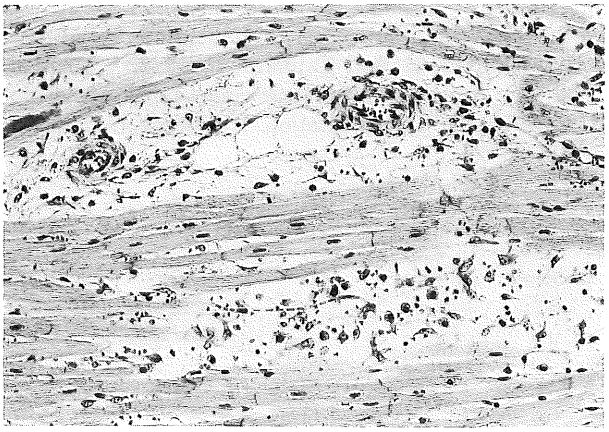

Fig. 5. Right ventricular specimen in acute rejection. (Hematoxylin and eosin stain ×160)
Extensive inflamatory infiltration in both perivascular and interstitial spaces and focal myocytelysis were found.

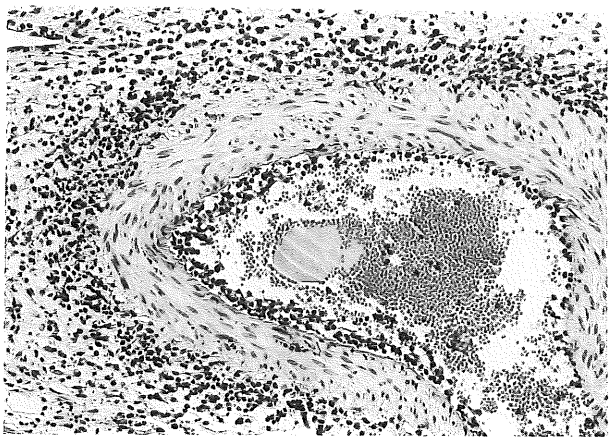

Fig. 6. Right ventricular specimen in the totally rejected heart. (Hematoxylin and eosin stain ×160)
Lymphocyle infiltration was observed in intima and adventitia of the intramural coronary arteries.

thickness increased to 9.8+0.5mm on day 3 and 10.5+0.8mm ($p<0.005$) on day 6 after transplantation (Fig. 2).

Endomyocardial biopsy was undertaken through the right jugular vein with 5 F. biotome for both donor and recipient hearts (Fig. 3). Microscopic examination of biopsy specimens obtained on day 2 revealed interstitial edema and minimal lymphocytic infiltrates (Fig. 4). Right ventricular specimens obtained by autopsy after total rejection showed extensive inflamatory infiltration in both perivascular and interstitial spaces and focal myocytolysis (Fig. 5). Moreover, lymphocyte infiltration was frequently observed in intima and adventitia of the epicardial and intramural coronary arteries (Fig. 6).

DISCUSSION

Carrel and Guthrie[2] reported the first cardiac transplantation in dogs in 1905, anastomosing the external jugular vein and carotid artery, vena cava, and on of the pulmonary veins of the donor heaert. Subsequently, Mann and associates[3] simplified these techniques by anastomosing the aorta to the internal carotid artery and the pulmonary artery to the external jugular vein. McGrough et al[4] devised a variety of methods for heterotopic cardiac transplantation including intrathoracic locations, such that the donor heart supported part or the whole of the recipient circulation. The present authors devised a heterotopic cardiac transplantation model which could maintain the whole of the recipient circulation[7].

However, one of disadvantages of the heterotopic cardiac transplantation is disability of endomyocardial biopsy under fluoroscopy, the latter being indispensable for diagnosis of acute cardiac rejection. This paper described a method of heterotopic cardiac transplantation in which endomyocardial biopsy can be easily performed, and which carries minimal morbidity. This technique has several advantages over heterotopic transplantation in the neck and abdomen. Problems of intraabdominal transplantation, including intussusception and intestinal obstruction, were avoided. The donor heart could be biopsied by passage of flexible cardiac biotome from the right internal jugular vein, through the superior vena cava, to the interior of the donor right ventricle like orthotopic cardiac transplants. In addition, biopsies could also be obtained from the recipient heart for comparison. This model may be used to study the various protocols of immunosuppression, and for non-invasive diagnostic evaluation to find rejection, such as echocardiography and electrocardiogram.

Echocardiography revealed that the posterior wall of the left ventricle at the end diastole progressively thickened. This increase was already observed by other investigators[5,6]. Popp et al[5] measured the thickness of the left ventricular posterior wall and demonstrated an increase during rejection. Sagar

et al[6] estimated left ventricular mass by M-mode echocardiography and pointed out a significant increase during rejection. This increase of wall thickness was due to myocardial edema caused by myocardial ischemia and acute rejection[6]. Histological analysis revealed that the initial change of the myocardium was interstitial edema lymphocyte infiltration around the small coronary arteries subsequently appeared.

Since this model is a non-functioning transplantation model, the process of acute rejection might be different from the orthotopic cardiac transplantation model. However, the pathological findings in this model closely resemble the microscopic findings of canine orthotopic transplants rejection reported by Billingam et al[1]. Therefore, this simple method of heterotopic cardiac transprantation might be useful in the evaluation of acute rejection and estimation of various immunosuppressive drugs.

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