Non-invasive Myocardial Strain Imaging to Evaluate Graft Failure in Cardiac Xenotransplantation

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Background: The shortage of human hearts for allotransplantation makes xenotransplantation a possible option for controllable organ providers. To detect acute xenograft rejection, invasive biopsy seems inevitable; however, this occasionally results in poor incision wound healing or infection. To date, no method of noninvasive imaging for early detection of xenograft rejection has been established. We hypothesized that ultrasound speckle tracking would better detect xenograft failure than routine left ventricular ejection fractions (EF).

Methods: From August 2013 to July 2015, a total of six cardiac heterotopic xenotransplants (α 1, 3-galactosyltransferase gene-knockout porcine heart) into cynomolgus monkeys were monitored with echocardiography every 3 to 7 days. M-mode and two-dimensional (2D)-EF measurements and myocardial strain analyses were performed. Cardiac xenograft pathology was reviewed from the immediate postoperative biopsy, as well as the necropsy.

Results: Myocardial speckle tracking analysis was feasible in all six cases. The longest survival was 43 days. Only one pathology-proven immunologic rejection occurred. Cardiac xenograft failure appeared as two types: a dilated pattern with decreased EF or a myocardial-thickening pattern with preserved EF. Both antibody-mediated rejection (n=1) and sepsis-induced myocardial dysfunction (n=2) revealed decreased radial or circumferential strains, but normal-range EF. Xenograft functional decline was significant with respect to radial or circumferential strain (P=0.028), but not to conventional M-mode or 2D-EFs (P=0.600, P=0.340, respectively).

Conclusions: Radial and circumferential strains were significantly decreased in both types of xenograft failure, regardless of EF. Further studies are warranted to correlate the strain analysis and immunopathological details.

Key Words: Heterologous transplantation, Heart transplantation, Echocardiography, Histopathology

INTRODUCTION

Progress in preclinical pig-to-non-human primate cardiac xenotransplantation is giving hope to future controllable organ providers in this era of a shortage of human hearts. One of the life-threatening barriers in pig-to-non-human primate xenotransplantation was the immunologic response of the recipient against the endothelial lining of the vasculature.
in the xenograft: hyperacute rejection has been controlled with α 1,3-galactosyltransferase gene-knockout (GT-KO) pigs expressing some human complement regulatory protein, and other acute humoral or vascular rejection has been overcome with various immunosuppressive regimens(1-3). Until now, heterotopic abdominal cardiac xenotransplantation has been a standard model to define genetic modification or immune suppression regimens which ultimately prolong graft contraction and perfusion without supporting monkey’s circulation. To detect the cardiac graft rejection, a catheter-derived right ventricular endomyocardial biopsy has been considered the gold-standard procedure. However, in the heterotopic small animal model, an invasive open biopsy on left ventricular (LV) seems inevitable, which subsequently can result in an increase of cardiac enzymes, poor incision wound healing, or infection. As xenograft survival increases, to detect early xenograft rejection during the prolonged period, non-invasive surveillance tools to avoid complicated serial invasive open biopsies seem essential to increase survival.

Echocardiography is a well-established, widely used non-invasive diagnostic tool to assess LV function. The conventional LV ejection fraction implies changes in the LV cavity dimensions or volumes regardless of LV wall mechanics—the ejection fraction or stroke volume itself would not be so important in this heterotopic non-life-supporting xenotransplantation model. Tissue Doppler or myocardial speckle-tracking strain imaging is a sensitive echocardiographic module to record tissue mechanics within the myocardium(4). Clinical and experimental studies with two-dimensional (2D) strain ultrasound imaging have been demonstrated a significant relationship between strain indexes and acute cellular rejection in posttransplant allograft surveillance(5-7), but this is still under discussion(8).

In this study, we investigate the feasibility of ultrasound myocardial speckle-tracking for heterotopic cardiac xenografts in monkeys from GT-KO miniature pigs, and whether myocardial strain would better reflect cardiac xenograft failure than routine M-mode or 2D LV ejection fractions.

MATERIALS AND METHODS

1. Experimental animals

From August 2013 to July 2015, we prospectively performed a total of six cardiac heterotopic xenotransplantations. The experimental animal model has been described previously(9). In brief, as a donor, we used homozygous GT-KO pigs (n=6, blood type A, 5 to 7 kg: Animal Biotechnology Division, National Institute of Animal Science, Suwon, Korea), as a recipient, we used cynomolgus monkeys (Macaca fascicularis, n=6, blood type A, 4 to 7 kg: Orient Genia Inc., Seongnam, Korea). The protocols were approved by the Orient Genia Institutional Animal Care and Use Committee (IACUC No. ORIENT-IACUC-11104).

2. Heterotopic cardiac xenotransplantation

Two cardiovascular specialty surgeons (J.S. Kim, H.K. Chee) performed heterotopic abdominal xenotransplantation as described previously(9). The donor pig ascending aorta root was anastomosed to the recipient monkey’s abdominal aorta, and the pig main pulmonary artery to the monkey’s inferior vena cava. The pig coronary arteries are perfused from the abdominal aorta, the coronary venous blood entering to the right heart via the coronary sinus, and then ejected into the inferior cava via the pulmonary trunk(10).

3. Immunosuppressive regimen

For recipients’ induction therapy, we used rabbit anti-thymocyte globulin (5 mg/kg/day for 4 days; Genzyme, Cambridge, MA, USA), rituximab (10 mg/kg/day for 2 days: Roche, Basel, Switzerland), cobra venom factor (0.05 mg/kg/day for 5 days), and anti-CD154 (20 mg/kg/day ×7; 5C8, provided by the NIH Nonhuman Primate Reagent Resource). For maintenance therapy, we applied FK 506 (by mouth at 4 mg/kg/day), mycophenolate mofetil (by mouth at 100 mg/kg/day), and methylprednisolone (intravenous at 2 mg/kg/day for 14 days, at 1 mg/kg/day for the next 7 days, and at 0.5 mg/kg/day thereafter).

4. Histopathologic analysis

For each the cardiac xenograft, we performed an open surgical biopsy immediately after heterotopic heart transplantation (postoperative day [POD] 0), and after expiry as
an autopsy. The specimens were fixed in 10% buffered formalin. The sections were embedded in paraffin, sectioned, and stained with hematoxylin and eosin (Dako diagnostics, Hamilton, CA, USA). Cell-mediated rejection was evaluated according to the International Society for Heart and Lung Transplantation Guidelines 2004(11). Acute antibody mediated rejection (AMR) was also assessed according to the International Society for Heart and Lung Transplantation guidelines with C4d immunostaining(11,12). Cause of death or complications were determined from autopsy as well as laboratory findings.

5. Echocardiography and myocardial strain analysis

We monitored the cardiac xenograft function via non-invasive echocardiography using the ultrasound LOGIQ platform (GE Healthcare, Waukesha, WI, USA), with a linear transducer (9 or 11 L) on immediate POD (POD 0), every 3 to 7 days thereafter, and immediately before expiry (POD X). Routine echocardiographic images include M-mode and 2D short axis views of LV at mid-level, and 2D apical four chamber views. LV dimensions and the ejection fraction were calculated from M-mode dimensions or the Teichholz formula and 2D Simpson's method(13). We defined the cardiac xenograft geometric changes as eccentric dilatation (increased LV mass and end-diastolic dimension) or concentric thickening (increased LV wall thickening and wall-to-cavity dimension ratio). LV mass was calculated from M-mode dimensions of LV mass (g)=0.8×[1.04 [(end-diastolic dimension + interventricular septal dimension + posterior wall dimension)3−end-diastolic dimension]]+0.6(14). We analyzed myocardial strain from the 2D short axis view of the LV at mid-level, on radial as well as circumferential peak systolic strain, using TomTec software (TomTec Imaging systems GmbH, Image-Arena platform, Fulda, Germany). Radial strain represents the percentage of radial thickening which is presented as positive values; circumferential strain, the percentage of circumferential shortening, as negative ones(15). The M-mode or 2D ejection fraction or myocardial strain was analyzed by an investigator blinded to the biopsy results.

6. Statistical analysis

We performed statistical analysis using IBM SPSS ver. 22.

| Table 1. Six cases of xenotransplantation: recipient survival and cardiac xenograft functional parameters |
|---------------------------------|-------------------|-----------------|-------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| LV remodeling                   | Immediate postoperative | Before expiry | Survival (day) | EDD (mm) | ESD (mm) | Wall (mm) | M-EF (%) | 2D-EF (%) | RS (%) | CS (%) |
| Eccentric dilatation            | 1                  | 4               | 7.50           | 3.98      | 4.80     | 83       | 78     | 4.80     | 10.79 | 13.75 |
|                                | 2                  | 9               | 5.36           | 4.46      | 5.21     | 41       | 35     | 0.166    | 0.057 | 26.08 |
|                                | 3                  | 43              | 7.84           | 5.10      | 6.24     | 70       | 60     | 3.26     | 0.126 | 29.77 |
|                                | 4                  | 38              | 18.21          | 13.04     | 5.07     | 45       | 45     | 5.40     | 0.225 | 5.96  |
| Concentric thickening           | 5                  | 9               | 17.52          | 13.63     | 6.11     | 48       | 45     | 1.100    | 0.419 | 3.78  |
|                                | 6                  | 9               | 22.58          | 19.22     | 4.92     | 34       | 32     | 1.832    | 0.057 | 14.71 |
|                                | 7                  | 43              | 7.64           | 5.10      | 6.24     | 70       | 60     | 3.26     | 0.126 | 29.77 |
|                                | 8                  |                | 18.21          | 13.04     | 5.07     | 45       | 45     | 5.40     | 0.225 | 5.96  |

Abbreviations: LV, left ventricular; EDD, LV end-diastolic cavity dimension; ESD, LV end-systolic cavity dimension; Wall, LV wall thickness; M-EF, M-mode LV ejection fraction; 2D-EF, two-dimensional LV ejection fraction by Simpson's method; RS, peak systolic radial strain; CS, peak systolic circumferential strain; ISHLT, International Society of Heart and Lung Transplantation; AMR, antibody mediated rejection.
Fig. 1. A donor heart (41.6 g) from a transgenic pig (α 1,3-galactosyltransferase gene-knockout, blood type A, 4-week-old, 6.7 kg) was transplanted into a cynomolgus monkey (blood type A, 5.5-year-old, 6.7 kg). Case number 3, pictured here, survived 43 days. (A, B) Hematoxylin and eosin staining of donor left ventricular walls at necropsy shows severe congestion, hemorrhages, myocyte necrosis, conspicuous endothelial cell changes, intravascular mononuclear cells (×400). No cellular rejection is present (International Society of Heart and Lung Transplantation [ISHLT] acute cellular rejection grade 0R). (C) Immunoglobulin G (IgG) staining of the donor left ventricular myocardium shows a positive stain in the necrotic area (×400). (D) Diffuse, multifocal capillary C4d staining with strong intensity of the corresponding specimen confirmed antibody-mediated rejection (ISHLT antibody mediated rejection) (×400).
Fig. 2. A donor heart (37.5 g) from a transgenic pig (α1,3-galactosyltransferase gene-knockout, blood type A, 6.4 kg) expressing human complement regulatory protein CD46 was transplanted into a cynomolgus monkey (blood type A, 5.3 kg). Case number 4, pictured here, survived 38 days. An M-mode tracing echocardiogram of the mid-level of the left ventricular (LV) short axis view compared with (A) immediate postoperative and (B) just before expiry. Two-dimensional echocardiograms of the mid-level LV short axis view at the end-systolic phase, (C) different from at the immediate postoperative, (D) just before expiry note the markedly thickened LV walls with a very small LV cavity. (E) Radial strain of the corresponding short-axis view measures 5.408% at immediate postoperative (F) decreasing to 0.050% just before expiry. (G) Hematoxylin and eosin staining of the cardiac xenograft LV walls immediately postoperative from an open biopsy shows a relatively normal myocardium (×400). (H) At autopsy (postoperative day 38), multifocal bacterial colonies with microabscesses (arrows) confirmed the sepsis-involved myocardium (×100).

**DISCUSSION**

This is the first heterotopic cardiac xenograft strain anal-
In a total of six cases, changes of echocardiographic parameters of the left ventricular (LV) dimension or function between immediate postoperative (postoperative day [POD] 0) and just before expiry (POD X) are compared using a Wilcoxon signed-rank test. Radial and circumferential strain show significant changes (P=0.028). Blue lines with round points represent LV eccentric dilatation; red dots with triangular points represents LV concentric thickening. (A) LV end-diastolic cavity dimension (EDD), (B) LV end-systolic cavity dimension (ESD), (C) interventricular septum (IVS), (D) LV posterior wall dimension (PWD), (E) M-mode LV ejection fraction by Teichholz’s method (M-mode EF), (F) two-dimensional LV ejection fraction by Simpson’s method (2D-EF), (G) radial strain, and (H) circumferential strain.

Fig. 3. In a total of six cases, changes of echocardiographic parameters of the left ventricular (LV) dimension or function between immediate postoperative (postoperative day [POD] 0) and just before expiry (POD X) are compared using a Wilcoxon signed-rank test. Radial and circumferential strain show significant changes (P=0.028). Blue lines with round points represent LV eccentric dilatation; red dots with triangular points represents LV concentric thickening. (A) LV end-diastolic cavity dimension (EDD), (B) LV end-systolic cavity dimension (ESD), (C) interventricular septum (IVS), (D) LV posterior wall dimension (PWD), (E) M-mode LV ejection fraction by Teichholz’s method (M-mode EF), (F) two-dimensional LV ejection fraction by Simpson’s method (2D-EF), (G) radial strain, and (H) circumferential strain.

ysis, to our knowledge, that has evaluated the xenograft failure. It was feasible to use strain analysis in all consecutive six cases. Progressive decrease of cardiac xenograft function was significant in radial or circumferential strain (P=0.028). Especially in cases of concentric remodeling of the LV (cases 3, 4, and 5), a small slit-like LV cavity gave a higher M-mode or 2D ejection fraction; however, the concentric thickened walls revealed significantly declined radial or circumferential strain (percent changes >50%), which emphasized the incremental diagnostic role of myocardial strains with an advantage of evaluating myocardial mechanics.

For the detection of cardiac xenograft rejection, several diagnostic tools have been applied in xenotransplantation, from manual palpations, blood sampling, and echocardiography, to the standard endomyocardial biopsy depending on the surgical design or experimental animals. Heterotopic abdominal cardiac xenograft survival can be monitored by the abdominal palpation score(16), which represents the superficial ventricle, mostly on the right ventricular free walls. With the advent of the internet, video surveillance of recipient activities or graft telemetric signals also has been available(17,18). Immunologic monitoring or biomarkers such as circulating organ-specific microRNAs have been implicated(19). Compared with indirect measurement of xenograft rejection, direct non-invasive visualization of graft contraction and understanding of myocardial mechanics seems promising for detecting early xenograft dysfunction or rejection, and this study demonstrated the feasibility of myocardial strain monitoring in pig to monkey heterotopic cardiac xenograft.
Clinically, in patients with orthotopic heart allotransplantation, 2D strain parameters have a demonstrated diagnostic role, and the European Association of Cardiovascular Imaging mandates measurement after heart transplantation(20). Mingo-Santos et al.(7) suggested that LV longitudinal strain measurement to exclude acute cellular rejection can reduce repeated endomyocardial biopsy. Indeed, real-world retrospective analysis used negative results to differentiate biopsy-proven cellular rejection during the first year after orthotopic heart transplantation(8). In an experimental model, radial strain seemed useful in early non-invasive detection of transplant rejection in a heterotopic rat cardiac transplantation model(5,6). Therefore, in this experimental animal study, we adopted the radial and circumferential strain from short-axis view of LV. Differing from the previous reports regarding the role of strain for allograft rejection(5-8) which were focused on the detection of “rejection” rather than “graft dysfunction,” this paper concentrated on xenograft dysfunction.

In this heterotopic xenograft study, the LV remodeling progressed into two distinct patterns: eccentric dilatation (n=3) versus concentric thickening (n=3). The eccentric dilatation cases tended to end with poor survival (less than 10 days). Among them, the cases with severe LV dysfunction (ejection fraction 10%, cases 2 and 6) had morphology similar to dilated cardiomyopathy; both M-mode and 2D ejection fractions well-appreciated the LV failure. In case 1, however, the conventional ejection fractions were within the normal range even at the time of sacrifice, but strain values were significantly decreased with the early detection of myocardial dysfunction. The percent change of strain in case 2 was less than 50% (−24% or −19%, respectively), because the immediate postoperative radial or circumferential strain was quite low with a suspicion of myocardial stunning, and before expiry was also low with severe LV dysfunction—the low absolute values of strain suggest myocardial contractile dysfunction. The concentric thickening cases tended to show better survival (cases 3 or 4, 43 or 38 days, respectively) with remarkable progressive concentric LV thickening. Histopathology of the thickened walls was noticeable with a substantial interstitial edema, hemorrhages and myocyte necrosis related with a C4d stain positive AMR in case 3 (Fig. 1), or mild interstitial edema, and multifocal microabscess related with sepsis involved myocardium in case 4 (Fig. 2). Abicht et al.(21) suggested that the LV wall hypertrophy was preclinical suspicion of humoral rejection or signs of thrombotic microangiopathy. In this small-number animal study with only one case of obvious AMR, it was difficult to confirm strain as early diagnosing xenograft rejection. But we could speculate that radial or circumferential myocardial strain suggested a diagnostic role in early xenograft dysfunction regardless of specific causes such as sepsis, ischemia or rejection. At the time of sacrifice, a failing LV with a low contractile or perfusion status might be depicted in lower strain indexes. As this study demonstrated, strain imaging better diagnosed LV contractile dysfunction than conventional M-mode or 2D ejection fractions in cases with progressive concentric remodeling. In concentric LV wall thickening, tissue Doppler derived indexes such as strain were able to depict subtle changes of LV wall mechanics or injury, at a time when conventional indices of LV ejection fractions remained normal or super normal due to small, slit-like LV cavity dimensions.

There are several limitations. First, the small number of cardiac xenografts for the 2 years of the study period led to it containing only one antibody-mediated rejection. Therefore more cases are needed to determine the diagnostic role of strain for xenograft rejection. Second, the fundamental limitation of myocardial strain analysis in cardiac xenografts is lack of a normal reference value of strain in miniature pigs. Therefore the authors demonstrate the percent changes instead comparisons with a normal value. Third, the higher heart rate, relative to humans, makes it more difficult to capture the proper peak systolic strain values, which difficulty could be overcome with better hardware or software with higher temporal resolution. Finally, along with the concentric thickening of LV walls, the end-systolic LV cavity size becomes closer to zero, limiting the application of Teichholz’s or Simpson’s formula, suggesting that another method is needed to evaluate LV function with thickened wall mechanics, such as measuring myocardial strain.

CONCLUSION

Non-invasive myocardial strain analysis in experimental
cardiac xenografts was feasible. Cardiac xenograft failure appeared as two types: a dilated pattern with decreased ejection fraction or a myocardial-thickening pattern with preserved ejection fraction. Radial and circumferential strains were significantly decreased in both types of xenograft failure irrespective of LV ejection fraction.

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